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American Journal of Veterinary Research

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INDEX NUMBER

A Publication of the
American Veterinary Medical Association

Volume IV OCTOBER, 1943 Number 13

Consecutive order of appearance, rather than identity with any given volume, is the plan adopted for numbering the issues of the American Journal of Veterinary Research. Following are the volume and issue numbers published to date.

VOL. I, No. 1—OCTOBER, 1940

VOL. II, No. 2—JANUARY, 1941
VOL. II, No. 3—APRIL, 1941
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VOL. IV, No. 10—JANUARY, 1943
VOL. IV, No. 11—APRIL, 1943
VOL. IV, No. 12—JULY, 1943
VOL. IV, No. 13—OCTOBER, 1943

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Published quarterly at 600 S. Michigan Ave., Chicago, Ill., by the American Veterinary Medical Association. Entered as Second-Class Matter January 31, 1941, at the Post Office at Chicago, Illinois, under the Act of March 3, 1879. Subscription price: $4.00 per year for United States, Canada, and countries affiliated with the Pan American Postal Union; foreign countries, $4.50. Members of the American Veterinary Medical Association may subscribe to the American Journal of Veterinary Research for $2.50 per year added to the annual dues of $7.00. Single copies $1.25. Address all correspondence to American Veterinary Medical Association.

American Journal of Veterinary Research

VOL. IV OCTOBER, 1943 NO. 13

Histological Changes in the Retina of the Vitamin A Deficient Horse

A. CLARENCE ANDERSEN, B.S., M.S. and GEORGE H. HART, V.M.D., M.D.

Davis, California

IN THE STUDY of vitamin A deficiency in the various species of farm animals at this station, 12 horses have been continued on vitamin A deficient diets until a fatal termination occurred, or they were killed when it was impending.

These experimental animals were used to demonstrate the syndrome of the deficiency (Howell, Hart, and Ittner[1]), to study the etiology of a rarefying condition found in the articular cartilages, which was proved not to be caused by this deficiency (Hart, Goss, and Guilbert[2]), and to ascertain the minimum requirements of this species for vitamin A (Guilbert, Howell, and Hart[3]).

In the course of the work, correspondence was carried on with Katherine Tansley of University College, London. At the Physiological Congress in Zurich in 1938, a personal discussion took place with her regarding certain phases of the study. Later, 1 eye from each of 3 vitamin A deficient horses and 1 from a normal horse were fixed in Zenker's fluid and sent to London for sectioning.

In order that the fixing fluid could quickly penetrate the posterior chamber, incisions were carefully made through the coats of the eyeball, exposing the vitreous humor. The fixed eyes were forwarded in 70 per cent alcohol colored with tincture of iodine. Tansley desired to obtain retinal sections from vitamin A deficient, large, domestic animal species. This was in connection with the elaborate studies being made by the University College group working on various phases of vitamin A deficiency.

While the outbreak of the war in 1939 interrupted the study, microphotographs, and stained sections of the retina of the first case (filly 2) in the work reported by Howell, Hart, and Ittner,[1] were returned to us.

A study of these sections and further correspondence with Tansley showed that, in the method of fixing the eyes, there was a separation of the retina at the junction of the outer segments of the rods and cones with the pigment epithelium. The latter remained attached to the choroid. The retinal sections from the vitamin A deficient horse showed ragged, irregular outer segments of the rods and cones. This was, without doubt, the result of the deficiency as Tansley had found this picture to be typical of the condition in her studies with other species. In this case, she cut the choroid separately and found that the ragged appearance of the rods was not due to their having torn away from the pigment layer. The equine retina is most delicate in this regard, as compared to the rat, dog, and cow. Special means of fixing must be used to prevent separation of the outer segments from the pigment cell layer.

MATERIAL AND METHODS

In this work, Bouin's, Pereny's, and Susa's formalin, and Held's and Zenker formol fixatives have been used. The latter gave the most satisfactory results. The first eye of each animal was enucleated immediately after death by severing the muscles and cutting through

From the Division of Animal Husbandry, College of Agriculture, University of California, Davis.

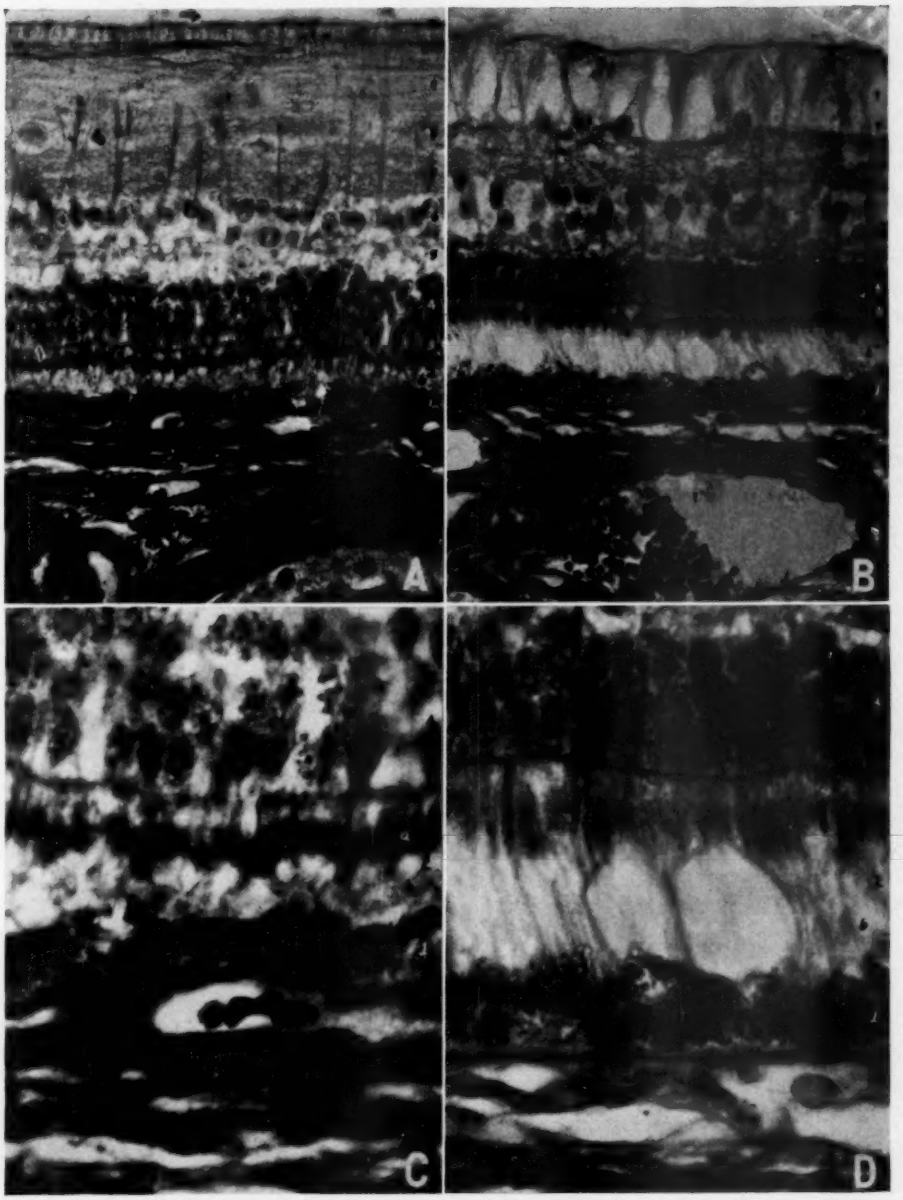

Plate I—Axial areas of normal and night blind vitamin A deficient horse retinas. Zenker formol fixative, Mallory azan stain.

Layers of the retina numbered in the figures: (1) Pigment epithelium. (2) Bacillary layer: (a) Rods and cones inner segments. (b) Rods and cones outer segments. (3) Outer limiting membrane. (4) Outer nuclear layer. (5) Outer plexiform layer. (6) Inner nuclear layer. (7) Inner plexiform layer. (8) Ganglionic cell layer. (9) Layer of optic nerve tracts. (10) Inner limiting membrane.

(See Captions on Opposite Page)

the optic nerve about 1 in. from its entrance into the eyeball. It was trimmed and immersed at once into the fixative. The second eye was then removed in the same manner and placed in the fixative.

A few minutes in the fluid allowed the sclera of the first eye to become somewhat hardened. The eye was then taken from the solution in order to inject fixative into the posterior chamber, while the vitreous humor was slowly extracted with a syringe. To accomplish this, a 16-gauge needle was inserted obliquely through the cornea and ciliary process into the vitreous humor. Fresh fixative solution was then syphoned from a raised flask through a rubber tube attached to the needle and passed directly into the posterior chamber. A second 12-gauge needle attached to a 50-cc. glass syringe was inserted through the optic nerve and papilla into the vitreous humor. The latter was then slowly withdrawn, at the rate of about 1 cc. per second, as the fixative siphoned into the posterior chamber from the other needle. Care was taken to keep slight pressure in the chamber and thus maintain the eyeball fully distended. This was continued until most of the vitreous humor was in the syringe, and fixative was flowing freely through the posterior chamber and into the syringe, so that the latter contained 40 to 50 cc. of vitreous humor and fixative. The two needles were then withdrawn and the eye returned to the fixative solution. This was repeated with the other eye, after which both were allowed to remain in the fixative until fixation was complete. Then, with the eye held in a horizontal position, an oval cap was removed by carefully cutting through the wall with a sharp scalpel. This exposed the anterior and posterior chambers. From this step, the entire eye or, preferably, cut pieces could be dehydrated, infiltrated with nitrocellulose, and sectioned. The nitrocellulose method is greatly superior to the paraffin method for delicate structures of this kind. With this technique, the retina could be kept intact in its normal position. For thin sections, it was necessary to carefully dissect off the sclera by gently cutting through the choroid after fixation was completed. A total of 20 horse eyes, mostly collected at a horse slaughtering establishment, as well as eyes from cows and dogs, were used in developing the technique.

In the critical study here reported, the eyes of 2 horses were used. The animals were a part of the experiment to determine the relation of vitamin A to joint lesions in this species.

They were maintained on an identical vitamin A deficient diet, except that the normal gelding received, in addition, 5 cc. twice weekly of high potency shark-liver oil, so prepared that it contained 75,000 I. U. (international units) of vitamin A per cc. This animal thereby received over twice the minimum requirements for significant storage, optimal dark adaptation, and reproduction, as determined by Guilbert, Howell, and Hart.[3]

The vitamin A deficient filly given no shark-liver oil gradually used up her storage and became night blind in 502 days. The method of testing horses for night blindness at this station has already been published.[3] She remained in this condition for 152 days and was killed after being on the experimental low A diet for 654 days. At the time of slaughter, the animal was in good physical condition and her blood serum showed a trace of vitamin A per 100 cc. From experience with other horses allowed to die from the deficiency, it was estimated she would probably have lived for several months. A piece of the liver, taken *post mortem*, was tested for the presence of vitamin A and found to be negative. The control gelding had been killed nineteen days previously, after having been on the experiment for 635 days. The blood of this animal showed 63 I. U. of vitamin A per 100 cc., and its liver contained 1,173 I. U. per Gm. of liver tissue. Thus, with a comparative study of the eyes of these 2 animals, any changes in the delicate structure of the retina due to the existence of rather prolonged night blindness could be recognized.

CAPTIONS FOR ILLUSTRATIONS ON OPPOSITE PAGE

Fig. A—Normal retina, including part of the choroid. x330. Note density of cells, compactness of bacillary layer and relative space comprising the outer segments of the rods and cones and the pigment layer.

Fig. B—Abnormal retina from vitamin A deficiency, including part of choroid. x330. Note the irregular outline of the pigment layer cells, relative space occupied by the outer segments of the rods and cones, and particularly the distortion of these processes by vacuolation.

Fig. C—Portion of normal retina, from outer nuclear layer to choroid. x1000. Note width of bacillary layer and relative size of outer and inner segments, as compared to these structures in figure D.

Fig. D—Portion of abnormal retina from vitamin A deficiency, from outer nuclear layer to choroid. x1000. Note the marked vacuolation in the area occupied by the outer segments of the rods and cones and their distortion.

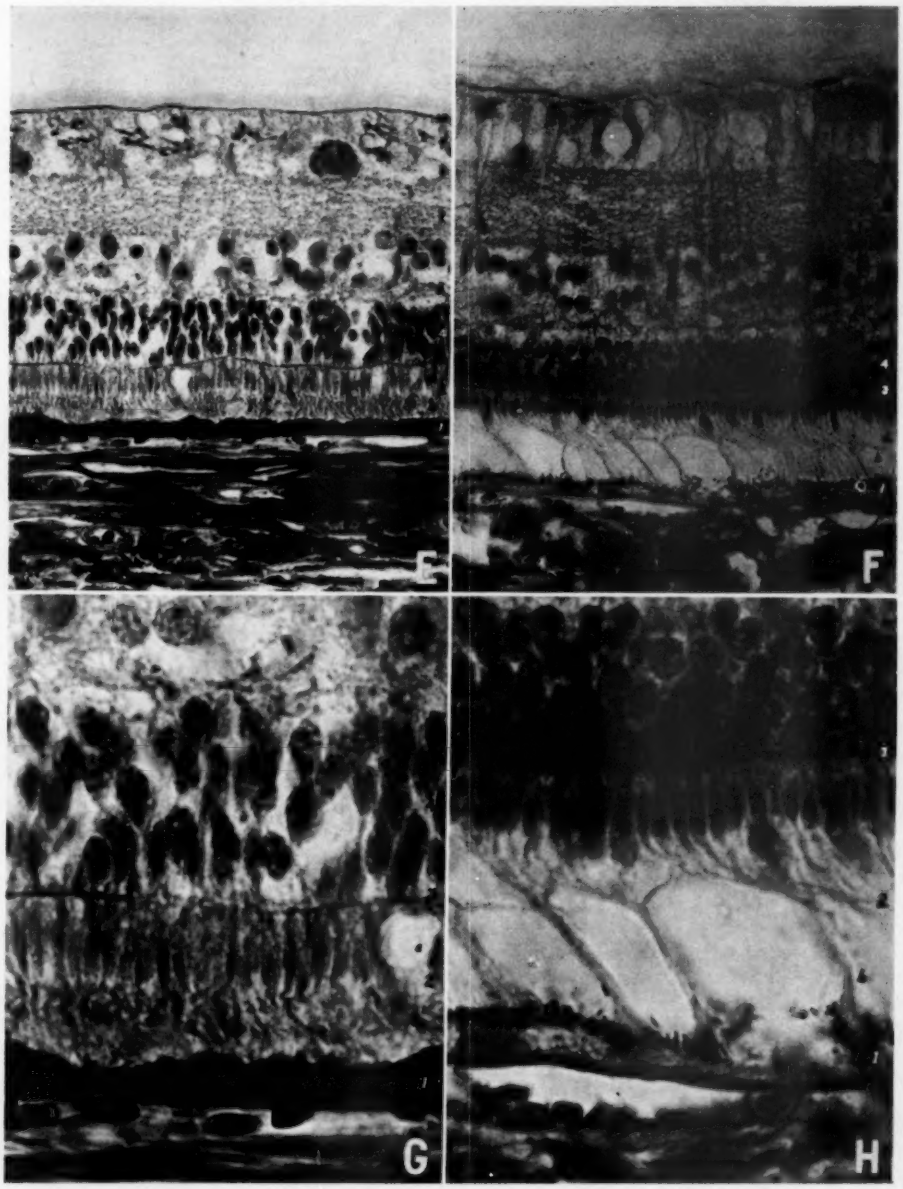

Plate 2—Peripheral areas of same horse retinas as shown in Plate 1. Same fixative and stain.

Layers of the retina numbered in the figures: (1) Pigment epithelium. (2) Bacillary layer: (a) Rods and cones inner segments. (b) Rods and cones outer segments. (3) Outer limiting membrane. (4) Outer nuclear layer. (5) Outer plexiform layer. (6) Inner nuclear layer. (7) Inner plexiform layer. (8) Ganglionic cell layer. (9) Layer of optic nerve tracts. (10) Inner limiting membrane.

(See Captions on Opposite Page)

HISTOLOGICAL STUDY

The equine retina is advantageous for the study of night blindness because of its size and relative structural simplicity compared to that of primates. In this species, all 10 layers of nervous tissue are present, but there is a reduction in the number of cells in the ganglionic and inner nuclear layers. A variation is observed in the layers of the normal equine retina, as in other species, according to the area under observation. Development is highest in the area immediately surrounding the medial axis. Toward the periphery, the development gradually recedes and there is a decrease in the number of cells in the ganglionic, inner, and outer nuclear layers, and in the size of the pigment epithelial cells toward the ora serrata.

The histological structure of the equine retina is, in general, more like that of species better adapted to night vision, as described by Walls.[4] Thus, there is reduced width of the retina from the outer nuclear layer to the inner limiting membrane, with relatively few ganglionic and bipolar cells.

For the critical study, sections were prepared of the respective retinas of the vitamin A deficient filly and the control normal gelding, using the same methods with tissues from the same areas of each retina. A variety of stains were used. The Mallory azan stain gave good differentiation of the layers and tissues, even though it is not the most precise nuclear stain. Iron hematoxylin was also satisfactory, giving a sharp nuclear stain but not such satisfactory differentiation of the layers. Sections varying in thickness from 2 to 12 μ were studied at magnifications of 400 to 3,420 diameters. Comparable sections of the retinas of the two horses showed distinctive alterations in the bacillary layer and the pigment epithelium of the vitamin A deficient animal (fig. A to J). There was a widening of the bacillary layer in the filly's retina due to the marked vacuolation (fig. B, D, F, and H). The pigment granules in the epithelial cells in the peripheral area were distinctly less numerous than in the same cells of the normal retina (fig. E to H). These changes, we believe, have to do with the characteristic green glare noticed in the eyes of horses, cattle, and dogs when they are in the advanced stages of vitamin A deficiency and night blindness has been present for some time. This is readily observed when looking through the pupil of the eye with the light coming over the shoulder of the observer directly into the eye of the animal.

In the vitamin A deficient retina, irregular unstained spaces or vacuoles were pronounced in the bacillary layer in both axial and peripheral areas (fig. B, D, F, and H). The outer segments of the rods and cones, while in direct apposition to the pigment cells of the normal retina (fig. A, C, E, and G), in many cases ended in the vacuoles in the affected retina. In the latter, there was an apparent lengthening of the outer segments, and bordering the large vacuoles, they accumulated into cords reaching toward the pigment cells (fig. D and H). This was accompanied by a reduction in the length of the inner segments of the rods, resulting in the corresponding segments of the cones standing out more prominently in the section of the affected horse (fig. F).

CAPTIONS FOR ILLUSTRATIONS ON OPPOSITE PAGE

Fig. E—Normal retina, including part of choroid. x330. Note the parallel arrangement of the rods and cones and their outer segments; also, the dense pigment and even arrangement of the pigment layer of cells.

Fig. F—Abnormal retina from vitamin A deficiency, including part of choroid. x330. Note the contrast with figure E, particularly the reduced density of the pigment cells and marked distortion and vacuolation of the outer segments of the rods; also, the projection of the inner segments of the cones beyond the rods.

Fig. G—Portion of retina of normal horse, from internal nuclear layer to choroid. x1000. Note the single vacuole in bacillary layer, denseness of pigment, and uniform contact of external processes with pigment cells.

Fig. H—Portion of retina from vitamin A deficiency, from external nuclear layer to choroid. x1000. Note scarcity of pigment granules in pigment layer as contrasted with figure G and the vacuolation distorting external processes of many rods into a single chord reaching toward the pigment cells.

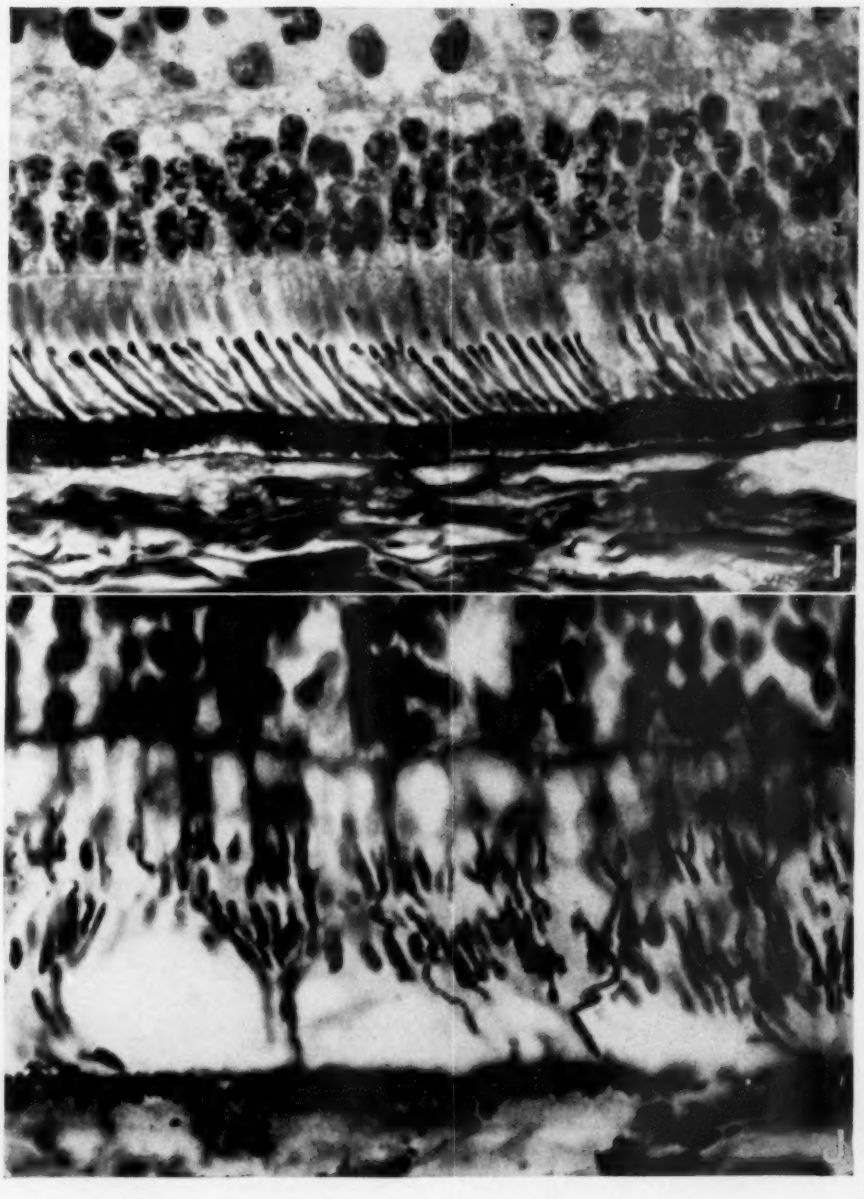

Plate 3—Peripheral areas of same horse retinas as shown in Plates 1 and 2. Modified Foot and Menard reticular stain after Zenker formol fixation. x1000.

Fig. I—Normal retina. Note the specific differential staining of the outer segments of the rods; also, their parallel arrangement and termination in the densely stained pigment cells.

Fig. J—Vitamin A deficient retina. Note the irregular broken outer segments, many of which do not reach to the pigment cells. They are prevented from doing so by the vacuolation in this area.

Layers of the retina numbered in the figures: (1) Pigment epithelium. (2) Bacillary layer: (a) Rods and cones inner segments. (b) Rods and cones outer segments. (3) Outer limiting membrane. (4) Outer nuclear layer. (5) Outer plexiform layer. (6) Inner nuclear layer. (7) Inner plexiform layer. (8) Ganglionic cell layer. (9) Layer of optic nerve tracts. (10) Inner limiting membrane.

In preparing the tissues and cutting through the wall of the eyeball after fixation, to obtain convenient sized pieces for sectioning, an abnormal amount of lipoid material exuded from the cut surface in the case of the A deficient animal. This was in the form of droplets, which floated on the surface of the 50 per cent alcohol solution in which the tissues were placed in starting to dehydrate them. We assume this lipoid material occupied the vacuole spaces in the bacillary layer. No eye tissue had been fixed in formol and, therefore, could not be stained with Sudan III.

It is interesting to note that pieces of the trachea of this horse were fixed in formalin. Lipoid material also exuded in cutting plugs of this tissue for sectioning. When these sections were stained with Sudan III, they gave the characteristic lipid staining reaction. The lipoid material was in the lamina propria adjacent to the epithelial layer, and no degenerative changes had taken place in the latter. This lipoid material was sufficiently characteristic in macroscopic appearance that it could readily be distinguished from adipose tissue and may be a manifestation of deficient vitamin A supply to epithelial structures. Josephs[5] reports decreased serum lipids in vitamin A deficiency with subsequent rise above normal animals when vitamin A was administered. This may be partially explained by the resorption of the excess lipids from the tissues.

With special silver and gold impregnation (modified Foot and Menard method), the structural relations between the outer segments of the rods and cones and the pigment cell layer could be demonstrated (fig. I and J).

In the normal horse, this showed the evenly stained, parallel, outer segments dipping into the pigment cells. In the vitamin A deficient retina, the staining of the outer segments was irregular, and many of them ended in the vacuoles in the bacillary layer. Observation is also directed to the deviation of the outer segments that do reach the pigment epithelium (fig. D, H, and J).

In an attempt to make a more detailed study of the relation between the outer segments of the rods and the pigment epithelium, special effort resulted in our obtaining very thin sections from the axial area that were 1 to 2 μ in thickness. Such sections could be very highly magnified. To accomplish this, the choroid had to be carefully dissected away under the binoculars, with the dehydrated piece of tissue in absolute alcohol, or after it had been hardened in nitrocellulose. Such a preparation from the vitamin A deficient retina is illustrated in text figure K. It is taken from an area between the vacuoles, in order to illustrate the position of the end processes of the outer segments. Careful examination of this section revealed that these processes did not merely touch the surface of the pigment epithelium, but dipped deeply into these cells. The ends within the epithelial cells appeared to be swollen into their clublike appearance probably from attempting to assimilate products from the pigment layer. Even at this high magnification, no degenerative changes could be seen in the outer nuclear layer of cells.

DISCUSSION

The present knowledge regarding the regeneration of visual purple in the light of the histological picture here presented may be discussed to possible advantage.

The duplicity theory of vision is based on the bleaching of visual purple in the light and its regeneration in the dark, constituting the so-called dark adaptation (Wald[6]).

Tansley,[7] in summing up the method of approach to a better explanation as to what actually occurs, states:

> It appears therefore that we have here at least three avenues along which we may seek information concerning the appearance of visual purple in the eye. We can inquire into the importance of the circulation and whether it has a specific function, *e.g.*, to remove waste products after bleaching, or whether it is only necessary for the efficient oxidation which will keep the retinal cells healthy. Or we can try to determine how close the connection between the outer limbs of the rods and the presence of visual purple really is. Lastly we can study the role of the pigment epithelium in regeneration.

It is now well established that the outer limbs of the rods contain the visual purple. This can be demonstrated by handling the tissues in dim light, to prevent complete bleaching, and fixing in platinum chloride. This stains the outer limbs of the rods yellow, and this reaction is due to the visual purple present in them. In studying the rôle of the pigment epithelium in the formation of the outer limbs and of visual purple, Tansley[7] found in rats that:

> The outer limbs which had developed in spite of separation from the pigment epithelium did not always look quite healthy and it was often difficult to make out their microscopic structure. In spite of this, however, their staining reactions were the same as those of a normal retina; they did not show those changes in color which were found to result from vitamin A deficiency. In all the eyes treated with platinum chloride after dark adaptation, the outer limbs were found to contain visual purple even when these had been developed inside rosettes.

The latter were obtained *in vivo* by trephining the sclera of the eyes of young rats while the retinas were still undeveloped.

The author felt these results tended to show that direct contact with the pigment epithelium was not a necessity for the regeneration of visual purple nor the normal histological development of the rods.

In our sections, we were impressed by the alterations in the outer segments and particularly their relation to the pigment cells. Despite the definite, differential staining of the outer segments in figures I and J, we have no histochemical data to show the presence or absence of any substance associated with vision, as the yellow color of platinum chloride is proof of the presence of rhodopsin. This method of staining definitely establishes that the condition of the outer segments described by Tansley[7,8] as unhealthy, ragged, or fraying exists in vitamin A deficiency. Also, many of these outer segments do not reach the pigment epithelium. From histological appearance, it is evident they were not functioning normally, and this was sustained by the clinical evidence that the animal from which the sections came was completely night-blind.

As we move to the peripheral area (fig. E to H) the vitamin A deficient retina showed marked reduction in the granules in the pigment epithelium. The so-called Purkinje phenomenon has established that a greater area of the retina is involved with night vision, and the pupil is dilated to bring a greater area of the retina into function. In the equine species, we allowed animals to die with vitamin A deficiency. Filly 2, in the experiments reported by Howell, Hart, and Ittner,[1] was such a case. This animal became night blind on June 15, 1937, 492 days after going on the deficient diet. She was given several periods of vitamin A treatment with alfalfa meal or cod liver oil in the study of minimum requirements. The animal continued to go down with the deficiency after this was discontinued and was killed *in extremis* on Sept. 16, 1938, 458 days after first showing night blindness. Sections of the retina showed no microscopic, degenerative changes internal to the bacillary layer.

This all would tend to show that pigment in the cells of the pigment layer and their contact with the outer segments of the rods was essential for normal night vision. The difference in the histological structure between this portion of the retinas of our vitamin A deficient and normal critical cases would show that here is the structural change occurring in night blindness. The fact that vacuolation is so extensive in the bacillary layer might be assumed to result from the accumulation of lipoid material in nature's effort to bring to these tissues the fat-soluble vitamin A. It would thus be a secondary change from the failure of the outer segments to get the necessary supply of vitamin A from the pigment cells into which their end processes so definitely dip.

The bacillary layer of the retina has been described as normally consisting of large amounts of lipoid material. Rhodopsin is a lipo-protein (Krause[9]), and the pigment epithelium contains the greatest amount of lipids of any layer in the retina (Krause[10]). The exact rôle vitamin A plays in normal night vision is still somewhat theoretical. In the recent work of Krause,[11] evidence

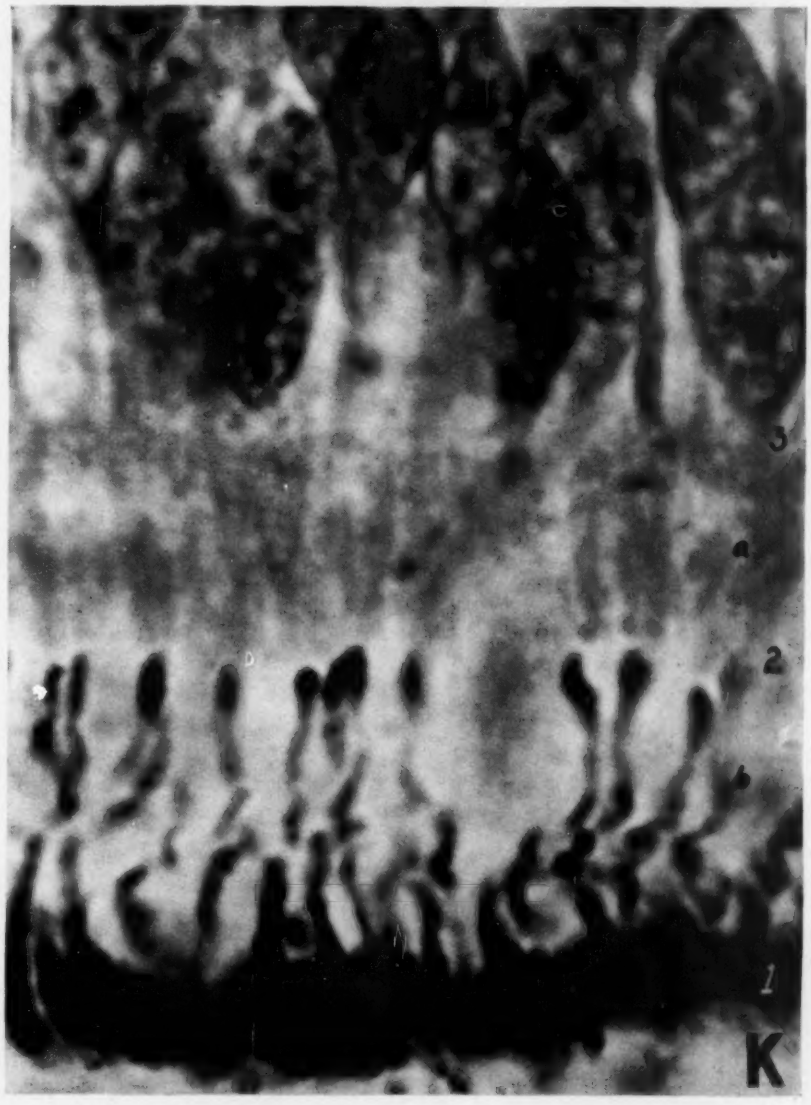

Fig. K—Vitamin A deficient retina. Modified Foot and Menard stain after Zenker formol fixation. Section, 2μ thick. x3420. Note the depth to which the outer segments of the rods dip into the pigment epithelium and their swollen, clublike ends.

Layers of the retina numbered in the figures: (1) Pigment epithelium. (2) Bacillary layer: (a) Rods and cones inner segments. (b) Rods and cones outer segments. (3) Outer limiting membrane. (4) Outer nuclear layer. (5) Outer plexiform layer. (6) Inner nuclear layer. (7) Inner plexiform layer. (8) Ganglionic cell layer. (9) Layer of optic nerve tracts. (10) Inner limiting membrane.

is presented that the production of rhodopsin is recognized to be dependent on the availability of vitamin A. In the case of deficiency of this essential, he believes the chromophoric group which appears to be linked with the lipid is not formed.

It is an accepted fact that the isolated retina cannot regenerate rhodopsin. The study of our sections tends to support the work of Hecht,[12] who states:

The regeneration depends to a large extent on material supplied by the circulation. Evidently the material in the pigment layer may be drawn on for the necessary ingredient in forming visual purple.

Johnson[13] noted in vitamin A deficient rats that the blood vessels throughout the

retina and choroid are greatly engorged with blood. Also, the connective tissue of the chorio-capillaris is noticeably increased in amount. Our sections showed clearly the close association of the capillary vessels in the choroid to the pigment epithelium (fig. E to H). There was also evidence of increased blood supply in the A deficient retina, as manifested by enlargement of the vessels and their engorgement with red blood cells (fig. A to H). This evidence would suggest the hypothesis that the increased blood supply results from the vitamin A deficiency. The pigment cells are taking lipids from the circulation in abnormal amounts in the effort to obtain the desired fat-soluble vitamin A. This excess is in turn taken up by the outer segments of the rods in their effort to get the necessary vitamin A to form rhodopsin. The excess lipid is filtered out of the rods into the intercellular spaces, which distort the outer segments. This results in the so-called fraying and ragged appearance of the rods, as well as the more ready separation of the bacillary layer from the pigment cells unless prevented by the use of special fixing methods such as we have described.

The Kolmer droplets, which have frequently been described as existing between the outer segments, are lipid material (Detwiler and Zwemer,[14] Walls[15]). Some of the droplets take the lipid stain with Sudan III, and Walls believes some of them are artifacts due to acetic acid used for their demonstration. They are more abundant in dark adapted eyes, but are not a part of the visual system. The older theory that these droplets came from the pigment cells has been replaced by evidence that they come from the outer segments of the rods (Kolmer,[16] Keeler[17]). These droplets probably account for the occasional vacuole in the normal retina (fig. E and G), and, in the high magnification, blur the area of the outer segments (fig. C).

When vitamin A therapy is supplied, the resorption of the lipoid material takes place, raising the blood lipids above that of normal animals, as mentioned by Josephs.[5] This gives opportunity for regeneration of the rods and for the retina to resume its normal structure, which accounts for the degree of recovery possible in these cases after prolonged night blindness has existed.

This is more marked in some species than in others. In sheep, permanent defects were commonly observed in vision detectable only in dim light which was permanent regardless of vitamin A therapy (Hart and Guilbert[18]).

SUMMARY

In the histological study of the night blind retina, the following structural changes were found. Widening of the bacillary layer with distortion of the outer segments of the rods and cones involving their contact with the pigment epithelium. This resulted from marked vacuolation in the area probably due to the accumulation of lipoid material. The pigment layer cells were also affected, particularly in the peripheral area of the retina, with reduced pigment granules and irregular surface.

These histological alterations seem to support the hypothesis that the outer segments receive the necessary ingredients for the production of rhodopsin from the pigment epithelium, which in turn receives them from the blood. In vitamin A deficiency, the large accumulation of lipoid material results from the effort of the rods to produce rhodopsin and is evidence that these lipids have passed from the blood to the pigment cells to the outer segments of the rods to the intercellular spaces between them.

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Rabies Virus in Mice

It has been generally assumed that rabies virus extends along the nerve paths to the central nervous system. This assumption is based on postmortem studies showing the localization of the virus at the time of death. The purpose of the following study was to ascertain the localization and dissemination of virus following various routes of infection in normal and immunized mice.

Using 3-week-old mice, one set received 0.5 cc. of 1 : 50 brain emulsion intraperitoneally, and the other set received 0.5 cc. of the same material subcutaneously. At stated intervals following inoculation, 2 infected mice were killed and various tissues examined for the presence of virus by intracerebral mouse inoculations (0.03 cc. of a 1 : 10 suspension of organs). Eight experiments were run with the peritoneal and subcutaneous method of infection. The results showed that, after intraperitoneal infection, virus could always be recovered from the peritoneum during the first six hours after the inoculation, but was no longer demonstrable after twenty-four hours. As a rule, virus could also be recovered from the spleen and liver during the first six hours, but not after twenty-hour hours. This was also true of the mesentery and peripheral lymph glands. Beginning with twenty-four hours, no virus could be detected in any organ or tissue tested until seventy-two to ninety-six hours after infection, when the virus was found only in the nerve tissues. It is of interest to note that after intraperitoneal infection, the earliest appearance of virus was always in the cervical cord and only subsequently in the brain and lastly in the lumbar cord.

Infection in the leg muscles gave somewhat different results. In this procedure, too, the virus was recoverable at the site of inoculation within four to six hours, but only once twenty-four hours after the infection. Immediately after the inoculation, there was no general lymphatic distribution as was the case with peritoneal inoculation. Only after the infection had progressed to paralysis (seven days) was virus recovered again at the site of inoculation as well as in the lymphatic glands. Apparently, after the virus has multiplied sufficiently, it spreads centrifugally. Of further interest is the first appearance of virus in the cord: in animals infected in the foreleg, the virus was first recovered from the cervical cord three days after infection; in those infected in the hind leg, the lumbar and thoracic segments of the cord were the first to be involved, also three days after infection.

In studying the effect of age on the path of distribution, it was noted that, using mice 3 weeks and 3 months old, the intraperitoneally inoculated virus disappeared from the lymphatics and visceral organs of both groups, to reappear on the fourth day in the cord in young mice, but not in the older ones. If, however, older mice succumbed to the infection, virus was found in the cord as in young mice.

The authors next directed their attention to the dissemination of virus in animals previously immunized with 1, 2, or 5 injections of 0.25 cc. of a formalized mouse brain or culture vaccine. The intraperitoneal test infection was given two weeks after the final immunizing dose, and only peritoneal washings, liver, and spleen were examined. In these immunized animals, the virus disappeared in three or six hours, depending on the degree of immunity. The experiment was repeated, using the subcutaneous routes for the test inoculations. The most consistent results were obtained with the mice receiving 5 immunizing doses. The rate of disappearance of virus from the site of subcutaneous inoculation depends on the degree of immunity. If immunity is not adequate to prevent the virus from reaching the cord, no difference is observed in the rate of spread to the other parts of the central nervous system in immunized as compared with normal mice; the mechanism of immunity consists apparently in a rapid destruction of the virus during the first few hours after infection.

[*I. J. Kligler and H. Bernkopf: The Path of Dissemination of Rabies Virus in the Body of Normal and Immunized Mice, Brit. J. Exp. Path., 24, (Feb. 1943): 653-658.*]

Book Review.

Staphylococcal Bovine Mastitis: I. Artificial Exposure of the Bovine Udder with Staphylococcus Aureus

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THE OCCURRENCE of staphylococci in the udder has been recognized ever since the inception of bacteriological study of bovine mastitis. Early reports associated the staphylococci, principally *Staphylococcus aureus*, with acute attacks of the disease and transitory habitation of the udder. In some cases, these acute attacks progressed to the gangrenous form with occasional fatal termination. More recent reports (1935-1940) indicate that staphylococcal mastitis is considerably more widespread than previously supposed and may, at times, constitute a herd infection (Gwatkin *et al.* 1936). Under such circumstances, the infection assumes a chronic form. The resulting changes both in the milk and the udder are indistinguishable chemically and physically from those induced by the streptococci.

Comparatively few references to artificial exposure of the udder with staphyloccoci have been found. Most of the reports deal principally with the injection of relatively large numbers of organisms through the teat canal. According to Munch-Petersen (1938), Kitt in 1891 produced slight inflammation in the udder by introduction of *Sta. aureus via* the teat canal. He also mentions the work of Wilkie and Gilruth in 1902 who injected *Staphylococcus albus* into 1 quarter with the production of chronic mastitis. Severe mastitis with systemic involvement followed injection of *Sta. aureus* into the udders of 2 cows and 3 heifers by Carpenter (1922). One cow died ten days later from staphylococcal septicemia. He used 2 cc. of a 24-hour culture recovered from a case of mastitis. Minett (1936) established *Sta. aureus* in 1 quarter with two exposures of 1 cc. each of a 24-hour culture, with an interval of nine days between exposures. The infection persisted for some months and into the next lactation period, after which it disappeared. The only record of the use of small numbers of *Sta. aureus* for exposure is by Little and Foley (1935). From 200 to 900 organisms were introduced into the teat on the small, beaded end of a glass rod on nine different occasions over a period of about five weeks. Acute mastitis appeared on the second day after the last exposure. The cow was destroyed ten days later because of general debility.

In the last few years, several dairy herds with a high incidence of staphylococcal infection of the udder have come to our attention. *Sta. aureus* was the principal offender. Chronic infection was present, and in a few herds, severe losses had occurred due to poor quality milk, acute attacks of the disease, and death of cows. These observations appeared to justify further study of *Sta. aureus* in the udder under controlled conditions. Accordingly, 8 lactating cows were exposed to this species by injection of small numbers into the teat just beyond the sphincter. Frequent examinations of milk samples from the exposed quarters were made to follow the progress of the infection.

EXPERIMENTAL

Of the 8 cows exposed to *Sta. aureus*, 2 (1997 and 2034) were 5 years old and had been used in other experiments. Prior to exposure, bacteriological examination of incubated milk samples from individual quarters of these animals failed to show staphylococci in any of them. After exposure, milk from the exposed quarters was exam-

From the Animal Disease Station, Bureau of Animal Industry, Agricultural Research Administration, U. S. Department of Agriculture, Beltsville, Md.

ined bacteriologically each week. The other 6 animals were in their first lactation period. Milk samples from each quarter were tested at weekly intervals from the time of parturition until the cow left the herd. These tests included the pH, chloride, catalase, Breed count, and bacteriological examination. The latter consisted of the Hotis test, microscopic examination of incubated milk films, and colony counts on poured blood-agar plates. Bovine blood was used exclusively. By these tests, all but 3 quarters of the 6 cows were normal at the time of exposure.

Two abnormal quarters occurred in cow 2514. A yellow, nonhemolytic coccus, that apparently was the cause of a high leucocyte count, had been present in the right front quarter since parturition. Both *Sta. aureus* and *Streptococcus dysgalactiae* were present in the secretion of the left front quarter three weeks prior to calving and both species persisted for about seven months, or until shortly before the cow was sacrificed. The left front quarter of cow 2484 became infected with *Streptococcus uberis* nine days before it was injected with *Sta. aureus*. An increase in both chlorides and leucocytes followed.

EXPOSURE

The technique of exposure was the same in each instance. After the quarter had been milked dry, the end of the teat was washed and thoroughly disinfected. One-tenth cc. of diluted staphylococcus culture was injected into the teat canal just beyond the sphincter with a sterile, 0.1-cc. syringe and a blunt-pointed 24-gauge needle. The injections were usually made in the morning and the cow was not milked again until ten to eleven hours later.

Actively growing cultures that had been transferred for several consecutive days were used. With few exceptions, the culture was incubated for eighteen hours at 37 C., diluted in 0.85 per cent salt solution, and injected immediately. At the time of dilution, poured blood-agar plates were made for colony counts. After exposure, 0.1 cc. of the dilution that was injected was spread on the surface of a blood-agar plate as a rough check on the number of organisms injected.

CULTURES

All of the strains of *Sta. aureus* used in this work, with the exception of strain 46, had been isolated from milk or abnormal udder secretions. This strain, known as Wood 46,* is frequently used as a toxin producer. Strain J4† was recovered from a milk sample with an average leucocyte count of about 4,000,000 per cc. At the time of isolation, the infection had been present in the udder for three months. The remaining 5 strains were isolated at the Animal Disease Station. Acute mastitis was present in the quarters from which strains 21A and 654C-1 were obtained. Both cows were in herds with many cases of staphylococcal mastitis. The status with regard to mastitis of the quarters from which 3 other strains were derived is not known, since the milk samples were examined bacteriologically only. Two strains (97A-1 and C64-2) were also from herds with extensive staphylococcal infection. The last strain (6HD-2) was isolated from an animal in a herd with little infection. All of the strains, with the exception of 46, were used within six months of isolation.

The cultures were all typical of *Sta. aureus* as indicated by morphology, chromogenesis, and action in differential mediums. They all produced characteristic alpha-beta hemolysis on bovine blood agar and coagulated rabbit plasma. The fermentation reactions and toxigenic properties of the cultures appear in table 1. Toxins were prepared by growing cultures in 0.3 per cent veal agar in the presence of 20 per cent carbon dioxide and 80 per cent oxygen for five days at 37 C. They were harvested by filtration through soft paper and Seitz filter. The filtrates were tested immediately or on the following day.

The toxigenic properties of the 7 strains are reasonably representative of those found in milk. A great deal of variation in the toxin-producing abilities of these

*Supplied by Lederle Laboratories, Inc.

†Supplied by Dr. J. M. Murphy, Dairy Research Station, New Jersey Agricultural Experiment Station.

TABLE I—Reaction of Staphylococcus Cultures in Differential Mediums and Tests for Toxicity

Culture No.	Fermentation Reactions*											Toxicity					Source of Culture
	Litmus Milk				M.B. Milk†		Lactose	Salicin	Mannite	Aesculin	Coagulase‡	Hemolysin§ titer	Lh dose (cc.)	Lethal‖ action in mice (Min.)	Dermonecrosis** (In.)	Enterotoxin††	
	A	C	R	P	C	R											
21A	+	+	+	—	+	+	S	—	+	S	+	1-2048	0.1	1½	1¼ x 2½	?	Cow's udder, acute mastitis
46	+	+	+	p	+	+	+	—	+	S	+	1-2048	0.06	½	1½ x 2	—	Man
J4	+	+	+	—	p	+	+	—	+	S	+	1-2048	0.12	1	1½ x 2	+	Cow's udder, acute mastitis
654C-1	+	—	S	—	—	S	+	—	+	S	+	1-4096	0.06	½	¾ x 3	—	Cow's udder, acute mastitis
97A-1	+	+	+	—	+	+	+	—	+	S	+	1-8192	0.06	3	1½ x 3	no test	Cow's udder
C64-2	+	S	S	—	—	S	+	—	+	—	+	1-128	0.0	none	slight reddening	no test	Cow's udder
6HD-2	+	+	+	—	S	S	+	—	+	—	+	1-8192	0.038	½	2 x 2	+	Cow's udder

A = acid; C = coagulation, R = reduction, P = proteolysis, + = positive, p = partial, S = slight, — = no action. Tests incubated for five days at 37 C. †Methylene blue milk 1:15,000. ‡Rabbit plasma diluted 1:4. Results observed after one hour and twenty-four hours incubation at 37 C. §Rabbit erythrocytes. End point taken at 50 per cent hemolysis. ‖Time required to kill white mouse with 0.1 cc. injected intravenously. **Area of necrosis measured forty-eight hours after injection of 0.1 cc. of toxin intradermally. ††Kitten test.

strains will be noted. Similar differences are found in testing cultures from the same herd and even from different quarters of the same udder. With the exception of strain C64-2, the toxins were very much alike with respect to the lethal and dermonecrotizing factors. On the other hand, there was a wide range in the alpha hemolysin content and Lh dose. Although only 4 strains were tested for beta hemolysin with sheep erythrocytes, similar differences were apparent with this fraction. Of these, strain 46 had the lowest titer (1:128), as would be expected since staphylococci from human sources are commonly found to produce little beta hemolysin (Carpenter, 1922). The highest titer (1 : 8192) occurred with strain 97A-1 and equaled the alpha hemolytic titer. With only 1 strain (C64-2) was there more beta than alpha hemolysin. The beta titer was 1 : 512, or four times that of the alpha.

RESULTS

One or more quarters of the udders of 8 cows were infected by the injection of small numbers of *Sta. aureus* into the teat canal. Some variation was found among the different strains of staphylococci in their ability to establish and maintain themselves in the udder. Differences also appeared in the susceptibility of the quarters to invasion by these organisms. With some strains, repeated exposure was necessary to bring about infection, and in these cases, a mild type of mastitis of a rather short duration developed. Conversely, with other strains, infection occurred after one or two exposures and chronic mastitis of a persistent nature was established. The results of the exposures are summarized in table 2.

Varying numbers of staphylococci were injected into 26 quarters on 55 occasions. Twenty-five exposures resulted in infection of 22 quarters. Of the 4 quarters that resisted infection, 1 (2491 RF*) was exposed once during the dry period, another (2484 LF) had two exposures, and the remaining 2 (2487 RF and LF) were each exposed three times. Three quarters (2484 RF and LR, and 2488 RR) were infected on two different occasions.

Three strains of staphylococci were used

* RF = right front quarter; LF = left front; RR = right rear; LR = left rear.

TABLE 2—Results of Exposure of the Udders of 8 Cows with 7 Strains of Staphylococcus Aureus

Cow No.	Quarter*	Strain No.	No. of Bacteria Injected	Interval Between Exposures (Days)	Duration of Infection (Days)	Results
1997	LF	21A	2000	—	53	Animal sacrificed after 53 days. Milk not tested for evidence of mastitis.
	RR	21A	1000	—	53	
2034	LF	21A	2000	—	101	Quarter dried up.
	RR	21A	2000	—	115	Milk not tested for evidence of mastitis.
2484	RF	21A	2200	—	43	Slight increase in leucocytes and chlorides.
	RF	21A	947	64	—	Infection not established.
	RF	21A	6370	14	—	Infection not established.
	RF	654C-1	tmtc†	160	60	Second lactation period. Slight increase in leucocytes and chlorides.
	LF	21A	6370	—	—	Infection not established.
	LF	C64-2	tmtc	195	—	Second lactation period. Infection not established.
	RR	21A	947	—	—	Infection not established.
	RR	21A	no count‡	21	—	Infection not established.
	RR	654C-1	tmtc	119	137	Second lactation period. Marked increase in leucocytes and chlorides.
	LR	21A	2200	—	—	Infection not established.
	LR	21A	1200	12	39	Slight increase in leucocytes and chlorides.
	LR	21A	947	57	—	Infection not established.
	LR	21A	no count	21	—	Infection not established.
	LR	97A-1	tmtc	187	82	Second lactation period. Marked increase in leucocytes and chlorides.
2486	RF	46	1174	—	378	Marked increase in leucocytes and chlorides.
	LF	46	tmtc	—	—	Infection not established.
	LF	46	2700	23	356	Slight increase in leucocytes and chlorides.
	RR	46	tmtc	—	—	Infection not established.
	RR	46	2700	23	356	Very marked increase in leucocytes and chlorides. One acute attack of mastitis.
	LR	46	1174	—	378	Marked increase in leucocytes and chlorides.
2487	RF	J4	no count	—	—	Infection not established.
	RF	J4	2380	15	—	Infection not established.
	RF	J4	700	8	—	Infection not established.
	LF	J4	no count	—	—	Infection not established.
	LF	J4	2380	13	—	Infection not established.
	LF	J4	700	8	—	Infection not established.
	RR	J4	2380	—	—	Infection not established.
	RR	J4	700	8	74	Slight increase in leucocytes and chlorides.
	LR	J4	no count	—	—	Infection not established.
	LR	J4	2380	8	168	Very slight increase in leucocytes and chlorides.
2488	RF	J4	700	—	—	Infection not established.
	RF	J4	780	12	—	Infection not established.
	RF	J4	4400	11	—	Infection not established.
	RF	J4	no count	46	—	Infection not established.
	RF	J4	2380	13	—	Infection not established.
	RF	J4	700	9	—	Infection not established.
	RF	6HD-2	tmtc	188	160	Second lactation period. Slight increase in leucocytes and chlorides.
	LF	J4	no count	—	—	Infection not established.
	LF	J4	2380	13	—	Infection not established.
	LF	C64-2	tmtc	188	159	Second lactation period. Marked increase in leucocytes and chlorides.
	RR	J4	no count	—	—	Infection not established.
	RR	J4	2380	13	35	Slight increase in leucocytes and chlorides. Infection lost during dry period.
	RR	97A-1	tmtc	188	159	Second lactation period. Marked increase in leucocytes and chlorides.
	LR	J4	700	—	114	No increase in leucocytes and chlorides. Infection did not persist through dry period.
2491	RF	6HD-2	tmtc	—	—	Exposed during dry period. Infection not established.
	LF	C64-2	tmtc	—	42	Exposed during dry period. Very slight increase in leucocytes.
	RR	97A-1	tmtc	—	108	Exposed during dry period. Marked increase in leucocytes and chlorides.
2514	RF	21A	947	—	24	Slight increase in leucocytes and chlorides.
	RR	21A	947	—	116	Very marked increase in leucocytes and chlorides.
	LR	21A	tmtc	—	—	Infection not established.
	LR	21A	637	22	103	Very marked increase in leucocytes and chlorides.

* RF = right front quarter; LF = left front; RR = right rear; LR = left rear. †Too many colonies to count on dilution plates. ‡Colony count was not made on this culture.

in the early part of this work. They were 21A, J4, and 46. Of the 3, only strain 46 of human origin established itself with any degree of regularity. Two quarters of cow 2486 became infected at the first exposure and the other 2 quarters at the second exposure. Typical chronic mastitis developed in the 4 quarters and the infection persisted through the dry state and into the succeeding lactation period. Strain 21A was but slightly pathogenic for cow 2484, with infection developing in 2 of the 4 quarters subjected to 10 exposures. Only slight changes were induced in these quarters and the infection was of short duration. Three other cows were infected more readily. Two quarters each of cows 1997 and 2034 and 3 quarters of 2514 were exposed eight times altogether with strain 21A. Staphylococci were established in 6 quarters with the first injection, and 1 quarter, the left rear of cow 2514, required a second exposure. Milk samples from the latter animal alone were examined for evidence of mastitis with the usual tests and the typical chronic form of the disease was observed. Four quarters of 2 cows (2487 and 2488) were exposed 21 times with strain J4. Infection occurred but once in the 2 rear quarters of each cow, and the changes that developed in the milk were mild in nature, although the organisms remained in 3 quarters for several months. Both the number of bacteria of the different strains injected and the time interval between injections varied considerably, as shown in table 2.

The 4 remaining strains of *Sta. aureus* were used in exposure of 3 cows. Two of these cows (2484 and 2488) had been exposed previously and were in the second lactation period. The third (2491) had completed its first lactation period and was exposed while dry, about two weeks prior to parturition. Three of the 4 strains of staphylococci (654C-1, 97A-1, and 6HD-2) were somewhat better toxin producers (table 1) than the 3 strains that were employed in the early exposures. Strain C64-2, however, exhibited the poorest toxigenicity of any of the strains.

With these 4 strains, 10 exposures of a like number of quarters in the 3 cows resulted in infection of 8 of them. This was a larger percentage of infection than had been obtained with the first 3 strains of staphylococci. Infection failed to follow injection of strain C64-2 into the left front quarter of cow 2484, or of strain 6HD-2 into the right front quarter of cow 2491. Of the 8 cases of infection, 3 were attended by slight changes in the composition of the milk, whereas in the remaining 5, the changes were typical of chronic mastitis. With one exception, the staphylococci remained in the udders for two to five months and longer. Infection was still present in 4 animals at the time of their removal from the herd five months after exposure, and no further opportunity was had to examine them. The 3 mild cases of mastitis were caused by different strains of staphylococci and occurred in 1 quarter of 3 cows (2484 LF, 2488 RF, and 2491 LF). Two strains (6HD-2 and C64-2) had failed to infect 1 quarter of each of 2 cows. On the other hand, the third strain (654C-1) and strain C64-2 induced typical chronic mastitis in 2 quarters of different cows. The fourth strain (97A-1) caused extensive changes in the milk of the 3 quarters into which it was injected. It may be noted that with the last 4 strains, the number of staphylococci injected was somewhat larger than in the earlier exposures. Although the cultures were not diluted sufficiently to permit accurate counting, it is estimated that about 30,000 organisms were injected in 0.1 cc.

DISCUSSION

As a cause of mastitis, the staphylococci have been regarded as subordinate to the streptococci. Recent evidence, however, indicates that *Sta. aureus*, at least, is assuming a rôle of increasing importance. It is evident, therefore, that additional knowledge concerning this species in relation to mastitis would be desirable. That *Sta. aureus* can invade the udder by way of the teat canal appears to be established by these results. The possibility of infection through other channels must be considered, but no information on this point has appeared thus far. To test the possibility of

hematogenous infection, 10 cc. of an eighteen-hour culture of strain 21A were injected intravenously into 1 cow. Six hours later, the animal displayed some difficulty in breathing and a temperature of 106.2 F. Clinical recovery was complete by the following morning, and repeated bacteriological examinations of the milk during the ensuing month failed to reveal *Sta. aureus* in any quarter. However, additional investigation would be necessary to prove definitely that the udder cannot be infected in this manner.

The differences in the ability of these strains of *Sta. aureus* to invade the udder and cause infection are characteristic of the vagaries of this species under natural conditions. In some herds, these organisms appeared occasionally in a few milk samples. On subsequent examinations, they might be found in the same quarters once or twice and then disappear, or they might be present in entirely different quarters. On the other hand, an entirely different situation existed in other herds. Staphylococci were present in milk samples from the same cows on repeated examinations over a period of a year or more.

Of the strains used for these exposures, strain J4 proved to be the least infective, whereas strains 46, 97A-1, and 654C-1 established themselves at the first injection. Three other strains (21A, 6HD-2, and C64-2) were somewhat irregular in their ability to infect the udder. The length of time the different strains remained in the udder could be correlated to some extent with their infectivity. That is, strains that infected the udder readily remained in the quarter longer than strains that required numerous injections. A somewhat similar relationship appeared with respect to the changes produced in the milk. Slight swelling of the quarter and flakes in the milk followed each injection, whether infection resulted or not. As a rule, these clinical manifestations subsided within a few days. When infection developed, the more infectious strains caused the more severe mastitis, as judged by the changes in the milk. Clinical mastitis appeared in only one instance. The right rear quarter of cow 2486 became slightly swollen about three months after the second calving and developed considerable induration. In mild cases of mastitis, an increase in the number of leucocytes constituted the only change in the milk. The more severe cases, on the other hand, were characterized by a high leucocyte count, marked increase in chlorides and pH. With the usual tests, these cases were indistinguishable from true cases of *Streptococcus agalactiae* infection.

In any consideration of an infectious disease, knowledge of the source of the infection as well as how the disease is transmitted and its effect on the host are of importance. There is little or no information available at present regarding the source of the staphylococci found in the udder. That they are more or less ubiquitous in nature and occur in many diseases in man and animals, such as infections of the skin and nasal and oral passages, is well known. It is, therefore, conceivable that these organisms could be introduced into the herd by a milker suffering from such infections. The ease with which strain 46 infected cow 2486 would appear to lend support to this theory. It is also possible, although definite proof has not appeared, that with the establishment of an active strain of *Sta. aureus* in 1 or 2 cows, the infection could be spread to other animals in the herd through milking. In that event, *Sta. aureus* infection, unlike *Str. agalactiae*, would present an ever-recurring hazard not easily controlled by the usual methods of segregation and sanitation.

SUMMARY

Six strains of *Staphylococcus aureus* that had been isolated from milk and 1 strain of human origin were used to expose 7 lactating cows and 1 dry one by way of the teat canal in an attempt to learn whether staphylococcal infection could be transmitted in this manner. Small numbers of organisms, varying from 700 to about 30,000, were injected into the teat canal just beyond the sphincter with a sterile, 0.1-cc. syringe and a small-gauge needle with a blunt point.

Staphylococci were injected into 26 quarters on 55 occasions. Twenty-five exposures resulted in infection of 22 quarters. One quarter of each of 2 cows and 2 quarters of a third cow were not infected after one, two, and three exposures respectively. Infection was established on two different occasions in 2 quarters of 1 cow and a single quarter of another animal.

The 7 strains of staphylococci displayed marked differences in their ability to infect and remain in the udder. The 4 quarters of 2 cows were exposed 21 times with 1 strain of *Sta. aureus* and the 2 rear quarters of both cows became infected. Mild cases of mastitis of short duration developed in these quarters. With 4 other strains of bovine origin, infection occurred in 8 of 10 quarters of 3 cows exposed but once. The resulting mastitis was typical of the chronic form of the disease and the staphylococci remained in the udder from two to five months and longer. One cow was exposed to the strain of *Sta. aureus* of human origin. Two quarters were infected with one exposure and 2 others acquired the infection after the second exposure. All 4 quarters developed chronic mastitis, and the only acute attack observed in the course of this work appeared in 1 of these quarters.

One cow was injected intravenously with an eighteen-hour bouillon culture of 1 of the strains of bovine origin. Staphylococcal infection was not observed in any of the 4 quarters.

The results of this work indicate that staphylococcal infection of the udder can occur by way of the teat canal. It would, therefore, be possible for *Sta. aureus* to be carried from an infected animal to an uninfected one on the hands of a milker, or on the teat cups of the milking machine. In addition, since the strain of human origin infected 1 cow with little difficulty, the possibility is suggested that *Sta. aureus* can be transmitted to the udder directly by an infected milker.

The Propagation of a Virus in Embryonated Chicken Eggs Causing a Chronic Respiratory Disease of Chickens

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IN RECENT YEARS, much progress has been made in the identification of the causative agents of respiratory infections of chickens. The information obtained has contributed materially to the control of some of these diseases and given information facilitating the identification of others that had not previously been differentiated.

The object of this report is to record the studies of a respiratory disease outbreak and of a virus which we believe has not been described heretofore.

This particular respiratory disease was observed in a flock of 5,000 laying birds. The disease was characterized by nasal exudation and respiratory râles and appeared to be of a rather slow-spreading type, persisting in the birds for weeks or months. The infection in this flock was first noticed in October 1941, and was brought to our attention in December. The owner stated that the birds had been in a good state of production prior to the development of the disease, but that in the pens showing the greatest degree of infection, egg production had dropped to an average of about 30 per cent and had persisted at that level. The disease had never been observed before on this poultry farm, and it is quite possible that it had been introduced with Barred Rock cockerels which had been purchased. The first cases occurred approximately two weeks after these birds had been placed in the breeding pens.

Clinically, the disease was similar to that observed in an outbreak of infectious bronchitis, but the duration of the infection seemed to preclude this possibility. The preliminary bacteriological examinations of the material submitted to the laboratory failed to indicate the presence of *Hemophilus gallinarum* or chronic fowl cholera organisms such as are sometimes found in birds showing a chronic respiratory disease.

The exudate from chickens affected with the disease under study resulted in infection of inoculated laboratory birds immune to infectious bronchitis and laryngotracheitis after seven and fourteen days. This served to differentiate between these diseases on the basis of their susceptibility and the period of incubation.

On the basis of the incubation period plus the bacteriological findings, infectious coryza due to *H. gallinarum* was eliminated as a possibility. In all of our experience, *H. gallinarum* infection is invariably noticed within twenty-four to forty-eight hours following artificial inoculation.

Since a disease of this type had previously been observed on several occasions, we visited the farm to make further observations under field conditions. Substantially the same symptoms were noticed as those which the owner had described, and upon observing the various pens, it was found that from 30 to 50 per cent of the birds were involved in those pens which had been infected for several weeks and fewer in those pens beginning to show trouble. The birds comprising the first pens in which the disease had been noticed in October had apparently recovered, as only an occasional bird appeared to be sick.

The most striking picture of the disease other than the nasal exudate and respiratory râles was the loss in body weight. The

Contribution 622 of the Rhode Island Agricultural Experiment Station; presented before the Section on Poultry at the seventy-ninth annual meeting of the American Veterinary Medical Association, Chicago, Aug. 24-27, 1942.

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mortality was low and did not appear of appreciable significance.

POSTMORTEM FINDINGS

In three birds submitted for examination, the postmortem findings of 1 bird examined at autopsy on December 16 and 1 December 19 failed to show anything other than a serous catarrhal type of nasal discharge, extensive mucous and catarrhal exudate in the trachea, and considerable congestion and edema of the lungs.

METHODS

The trachea was removed from the bird examined on December 16 and mucus from the lower region obtained, as described by Delaplane,[1,2] for the isolation of *H. gallinarum* and infectious bronchitis virus for egg-propagation purposes. By this technique, *H. gallinarum* was excluded as the cause of the trouble in this case.

Two Rhode Island Red pullets, immune to both infectious bronchitis and laryngotracheitis, were inoculated with the exudate from the trachea and nasal passages of the affected bird. Neither one showed any symptoms until after seven to fourteen days. The cultures failed to show any growth after twenty-four hours, or several days incubation, and thus it seemed probable that the infection was not produced by *H. gallinarum*.

Material from the lower trachea was re-

TABLE 1—Results of Inoculating 11- to 15-Day-Old Embryos with the Virus of a Chronic Respiratory Infection of Chickens

Passage No.*	Interval†	Age of Embryos (Days)	No. of Eggs‡	No. Dead§ (Days following inoculation) 1st	2nd	3rd	4th	5th	6th	7th	8th	9th	Remarks
1	0‖	11	I-8	0	0	0	0	0	0	—	—	—	All harvested for 2nd passage; no changes noted.
			C-10	0	0	0	0	0	0	—	—	—	
2	7	12	I-8	0	0	0	0	—	—	—	—	—	Harvested for 3rd passage; no changes noted.
			C-10	0	0	0	0	—	—	—	—	—	
3	7	12	I-6	2	2	0	0	0	0	—	—	—	Dead embryos hemorrhagic. Liver and kidneys congested and enlarged.
			C-10	0	0	0	0	0	0	—	—	—	
4	3	14	I-7	6	1	—	—	—	—	—	—	—	Embryos hemorrhagic; enlarged, congested liver and kidney. Used to inoculate birds Jan. 7, 1942.
			C-10	0	0	—	—	—	—	—	—	—	
5	4	12	I-8	1	2	0	0	0	0	—	—	—	Dead ones hemorrhagic. Live embryos showed whitish foci on chorioallantoic membrane.
			C-10	0	0	0	0	0	0	—	—	—	
5	6	14	I-8	4	0	0	—	—	—	—	—	—	Dead ones hemorrhagic as in passage 4. Two live ones examined 3rd day showed whitish foci on chorioallantoic membrane.
			C-10	0	0	0	—	—	—	—	—	—	
6	2	14	I-4	0	0	0	0	0	—	—	—	—	Harvested; whitish foci on chorioallantoic membrane.
			C-10	0	0	0	0	0	—	—	—	—	
7	3	12	I-4	3	0	0	0	0	0	—	—	—	Dead embryos hemorrhagic; congested kidneys.
			C-10	0	0	0	0	0	0	—	—	—	
8	2	14	I-6	1	0	0	0	—	—	—	—	—	Lesions noted only in dead ones.
			C-10	0	0	0	0	—	—	—	—	—	
9	1	15	I-7	4	0	0	—	—	—	—	—	—	Dead ones hemorrhagic; enlarged kidneys. Live ones, no lesions.
			C-10	0	0	0	—	—	—	—	—	—	
10	2	10	I-6	2	0	0	—	—	—	—	—	—	Harvested after 3 days; no lesions noted.
			C-10	0	0	0	—	—	—	—	—	—	
11	1	11	I-6	1	0	0	—	—	—	—	—	—	Harvested after 3 days; no lesions noted.
			C-10	0	0	0	—	—	—	—	—	—	
12	1	12	I-8	0	0	0	0	0	1	0	1	—	Dead embryos smaller than normal; show enlarged kidneys with retained urates. Remainder ready to hatch.
			C-10	0	0	0	0	0	0	0	0	0	
13	1	13	I-4	2	0	0	0	0	0	0	—	—	Live ones hatched. Dead ones hemorrhagic; congestion and enlargement of kidneys.
			C-10	0	0	0	0	0	0	0	—	—	
14	1	14	I-8	5	0	0	0	0	0	—	—	—	Dead ones hemorrhagic; congestion and enlargement of kidneys. Live ones ready to hatch.
			C-10	0	0	0	0	0	0	—	—	—	
15	1	15	I-5	1	0	2	0	0	2	—	—	—	Embryos smaller than controls. Liver, whitish foci. Kidneys enlarged, retained urates.
			C-10	0	0	0	0	0	0	—	—	—	
16	4	11	I-6	0	0	0	0	0	1	2	2	—	Two dwarfed embryos; urate deposits all internal organs. Kidneys grossly enlarged.
			C-10	0	0	0	0	0	0	0	—	—	
17	6	11	I-10	3	0	0	0	0	0	1	2	—	Dead embryos smaller than controls; mottled enlarged liver, kidneys enlarged. Whitish foci on chorioallantois.
			C-10	0	0	0	0	0	0	0	0	0	

*All of the various virus passages indicated in this table received 0.2 cc. of virus excepting 5, 6, 7, 10, and 25, which were injected with 0.5 cc. of virus.
†Interval in days between passages. ‡I = Inoculated eggs; C = Control eggs. § — = Eggs harvested and no further observations made. ‖Date of first passage (Dec. 19, 1941).

moved aseptically from the bird examined on December 19 for egg-propagation purposes. The mucous exudate was suspended in sterile 2 per cent sodium citrate-normal salt solution. It was divided into two portions, one for cultural purposes to test for bacteriological sterility, and the other for use in inoculating 8, 11-day embryonated chicken eggs. The eggs were inoculated without awaiting the outcome of bacteriological tests because it was decided that in case of bacterial contamination they could be discarded. The technique of egg inoculations and harvesting was similar to that employed by Deleplane and Stuart[2] for cultivation of infectious bronchitis virus. The bacteriological tests failed to show any growth in the material obtained for the egg passages.

The remaining bird was kept under observation to determine how long it would continue to show the infection. The 2 laboratory-inoculated birds were kept under observation to determine the incubation period and duration of infection.

The first eggs were harvested on the sixth day following inoculation and the chorioallantoic membranes removed aseptically to sterile test tubes for further egg propagation purposes. No indications that growth had occurred could be detected by daily candling of the eggs, nor from appearances of the embryos or the chorioallantoic membranes. The bacteriological tests of the harvested membranes failed to show any growth.

The membranes from eggs of the second

TABLE 1—Results of Inoculating 11- to 15-Day-Old Embryos with the Virus of a Chronic Respiratory Infection of Chickens

(Continued)

Passage No.*	Interval†	Age of Embryos (Days)	No. of Eggs‡	No. Dead§ (Days following inoculation) 1st	2nd	3rd	4th	5th	6th	7th	8th	9th	Remarks
18	15	12	I-10	2	1	0	0	0	0	0	2	—	Kidneys enlarged, urate retention; whitish foci on chorioallantoic membrane.
			C-10	0	0	0	0	0	0	0	0	—	
19	7	12	I-8	1	0	0	0	0	0	—	—	—	Kidneys enlarged of those harvested 6th day.
			C-10	0	0	0	0	0	1	—	—	—	
20	9	14	I-8	2	0	0	0	1	—	—	—	—	24 hr. dead embryos hemorrhagic, congested kidneys. Live ones hatched.
			C-10	0	0	0	0	0	—	—	—	—	
21	4	11	I-10	4	0	0	0	0	0	0	0	0	Dead ones hemorrhagic; enlarged kidneys. Remainder ready to hatch.
			C-10	0	0	0	0	0	0	0	0	0	
22	1	12	I-10	2	0	0	0	0	0	0	0	0	Dead ones hemorrhagic, others ready to hatch.
			C-10	0	1	0	0	0	0	0	0	0	
23	2	14	I-8	2	1	0	0	0	1	—	—	—	Dead ones hemorrhagic. 20th day dead ones enlarged kidneys, urate retention.
			C-10	0	1	0	0	0	0	—	—	—	
24	4	11	I-8	0	1	0	0	—	—	—	—	—	Dead ones hemorrhagic; others harvested.
			C-10	0	0	0	0	—	—	—	—	—	
25	1	12	I-8	4	0	0	0	0	0	2	—	—	Dead ones hemorrhagic; 2 dead on 19th day, show enlarged kidneys.
			C-10	0	0	0	0	0	0	0	—	—	
26	2	14	I-8	4	0	0	0	0	0	—	—	—	Dead ones hemorrhagic; 4 others ready to hatch.
			C-10	0	0	0	0	0	0	—	—	—	
27	4	11	I-8	1	0	1	0	0	0	0	0	1	Dead ones on 20th day enlarged kidneys, whitish foci on chorioallantoic membrane; 3 hatched, 3 died during hatching.
			C-10	0	0	0	0	0	0	0	0	0	
28	2	14	I-8	2	0	0	0	0	0	1	1	—	Live ones hatched. Others characteristic lesions.
			C-10	0	0	0	0	0	0	0	0	—	
29	5	12	I-8	4	1	0	0	0	0	0	1	—	Early dead ones hemorrhagic, enlarged kidneys. None hatched although shell pipped.
			C-10	0	0	0	0	0	0	0	0	—	
30	2	14	I-4	1	0	0	0	0	1	—	—	—	Dead ones characteristic. None hatched but pipped the shell.
			C-10	0	0	0	0	0	0	—	—	—	
31	4	11	I-8	1	0	0	0	0	—	—	—	—	One dead on 17th day; smaller urate retention.
			C-10	0	0	0	0	0	—	—	—	—	
32	3	14	I-8	0	0	0	0	0	0	—	—	—	Live ones hatching.
			C-10	0	0	0	0	0	0	—	—	—	
33	1	15	I-8	2	1	0	0	0	—	—	—	—	Live ones hatching.
			C-10	0	0	0	0	0	—	—	—	—	
34	3	11	I-8	2	0	0	0	0	0	0	1	—	One dead on 20th day; smaller than controls; kidneys enlarged, urate retention.
			C-10	0	0	0	0	0	0	0	0	—	
35	3	14	I-8	1	2	1	0	0	0	—	—	—	Live ones ready to hatch.
			C-10	0	0	0	0	0	0	—	—	—	

*All of the various virus passages indicated in this table received 0 2 cc. of virus excepting 5, 6, 7, 10, and 25, which were injected with 0.5 cc. of virus.

†Interval in days between passages. ‡I = Inoculated eggs; C = Control eggs. § — = Eggs harvested and no further observations made. ‖Date of first passage (Dec. 19, 1941).

passage were harvested after four days of incubation, and they too had remained normal so far as could be observed.

Two of the 6 embryos of the third passage died within twenty-four hours and 2 more died within forty-eight hours. The material harvested from these eggs proved to be sterile, as indicated by bacteriological examinations. The dead embryos showed extensive hemorrhages of the skin and congestion and enlargement of the liver and kidneys. These findings constituted the only suspicious lesions of growth of an infectious agent, as the remainder of the embryos appeared normal.

The technique for the subsequent egg passages was similar to that described, except that in some instances, 11-, 12-, 13-, 14-, and 15-day-old embryos were used for inoculation. In some lots of eggs, the amount of the inoculum varied from 0.2 to 0.5 cc. per egg in an effort to determine whether the amount of inoculum had any effect in explaining the death of the embryos within twenty-four hours after inoculation. Material harvested from embryos dying after twenty-four hours and from eggs incubated for several days was also used to compare the effect this might have on the concentration of the virus.

Egg-propagated material was used to inoculate mature and younger birds to determine whether the disease could be reproduced from such material. Daily observations were made to determine the incubation period and duration of the infection.

No filtration studies have thus far been conducted, and our criteria for the presence of a virus were based on inability to demonstrate bacterial growth in cultures nor organisms in stained preparations of the egg material.

RESULTS

Continuous Passage in Egg.—A summary of 35 egg passages is shown in table 1. It will be seen that the results are somewhat irregular, but that there is a general trend for the virus to kill embryos within a period of twenty-four hours in from 25 to 50 per cent or more of the inoculated eggs. In those embryos surviving more than twenty-four hours, an occasional one died on succeeding days, but the bulk of them lived through to hatching time. No noticeable effect on size could be detected from candling the eggs except in isolated instances where an embryo was slightly smaller than normal. The use of 11-, 12-, 13-, 14-, and 15-day-old embryos gave results which seem to indicate that embryos at least 14 days of age are perhaps the most satisfactory for growth of the virus. The fact that the virus grew in embryos of this age seems to exclude the relationship of this disease to that described by Nelson[3,4] as occurring from a coccobacilliform infection.

TABLE 2—Results of Using 5-, 6-, 7- and 8-Day Harvested Embryo Virus for Inoculating 12- and 14-Day Embryos

Age of Virus Eggs (Days)	Age of Embryo (Days)	No. Eggs	No. Dead Days following inoculation									Remarks
			1st	2nd	3rd	4th	5th	6th	7th	8th	9th	
6	12	i-8	0	0	0	0	0	—	—	—	—	Passage 2, virus.
		c-10	0	0	0	0	0	—	—	—	—	
5	14	i-12	1	2	0	0	0	—	—	—	—	Virus 2b.
		c-10	0	0	0	0	0	—	—	—	—	
7	14	i-10	2	0	0	0	5	2	2	—	—	Virus 5b.
		c-10	0	0	0	0	0	0	0	—	—	
8	14	i-10	2	0	0	0	0	1	—	—	—	Virus 9b.
		c-10	0	0	0	1	0	0	—	—	—	
6	14	i-8	0	0	0	0	0	0	4	1	—	Virus 23a.
		c-10	0	0	0	0	0	0	0	0	—	
7	14	i-8	4	1	0	0	1	0	0	0	—	Virus 25a.
		c-10	0	0	0	0	0	0	0	0	—	
8	12	i-10	2	1	0	0	0	0	0	2	—	Passage 18 virus.
		c-10	0	0	0	0	0	0	0	0	—	
7	14	i-8	2	0	0	0	1	—	—	—	—	Passage 20 virus.
		c-10	0	0	0	0	0	—	—	—	—	

— = Eggs harvested and no further observation made.
i = Inoculated eggs.
c = Control eggs.

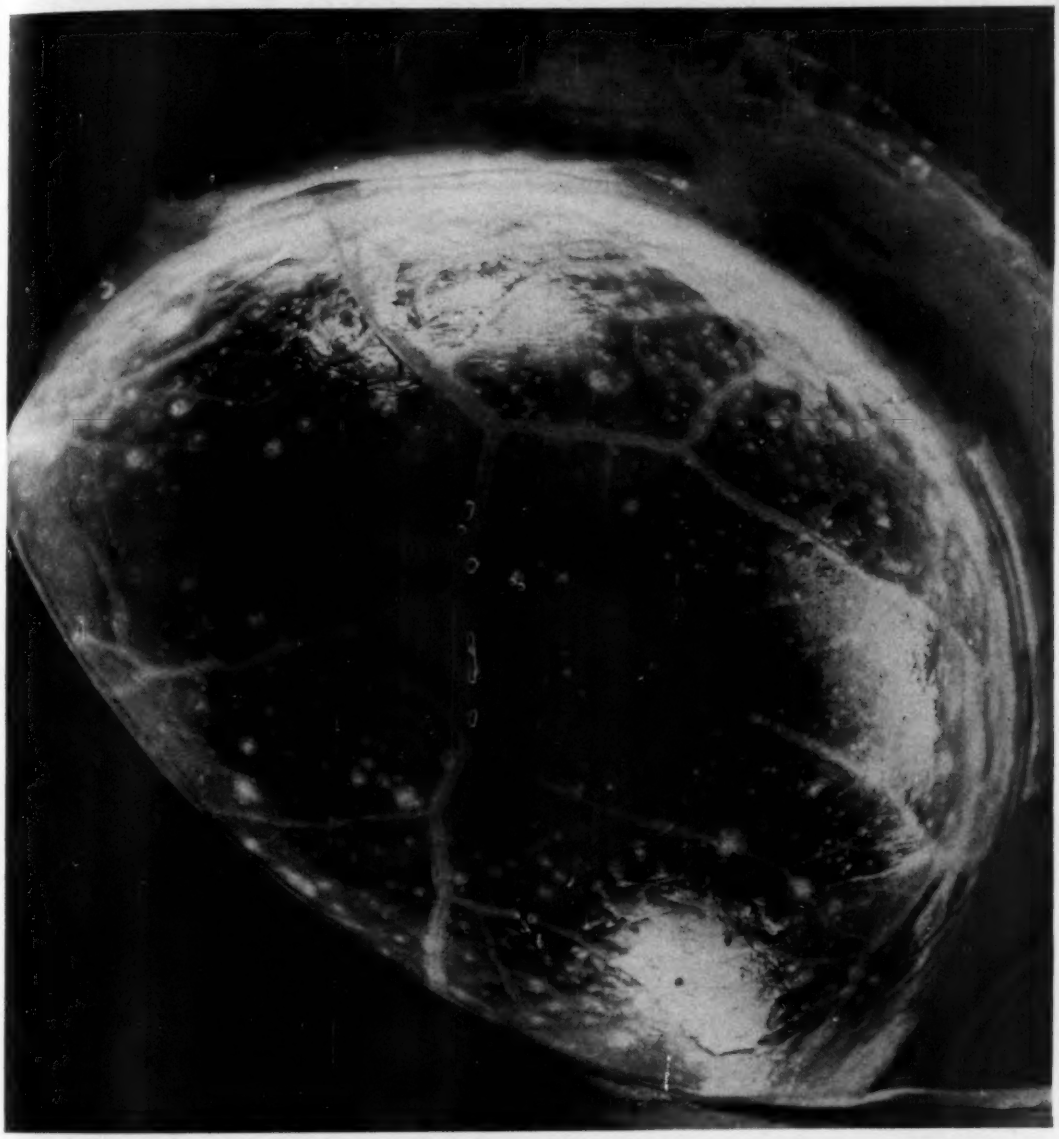

Fig. 1—Whitish foci produced by the virus on the chorioallantoic membrane after five days incubation. The lesions are extensive in this instance, but most membranes failed to show as many lesions as are visible here.

Material harvested from the embryos after five, six, and seven days of incubation did not seem to have a virus concentration any greater than that harvested from embryos which died after twenty-four hours of incubation.

The amount of the inoculum did not appear to be a factor in causing the early embryo deaths, as this seemed to vary with the material from different egg passages in which the concentration of virus no doubt varied (table 2).

After the first 4 transfers, no appreciable increase in virulence was observed in the remainder of the passages. This is in marked contrast to the virus of infectious bronchitis which, after 10 to 12 transfers, so affects the embryo size that the infected

embryos may be detected by candling (Beaudette,[7] Delaplane[2]).

Freshly harvested material seemed to be superior for inoculation purposes to fresh virus frozen and held for several days before being used. Virus held in the frozen state for two weeks in the electric refrigerator remained viable, but the maximum period of survival has not been determined.

Lesions present in embryos dying after twenty-four hours consisted of extensive hemorrhages of the skin and marked congestion, enlargement, and hemorrhages of the liver and kidneys. No lesions were seen in the chorioallantoic membranes in such instances.

When embryos died two or more days following inoculation, the lesions of the chorioallantoic membrane consisted of whitish, circumscribed foci which sometimes coalesced, extending to areas other than where the material had been injected (fig. 1). The embryo lesions were characterized by enlargement of the kidney and urate retention.

In those embryos which approached the state of hatching, the kidney changes were similar to those already described. There was a tendency for the embryos to pip the shell and fail to hatch, while a large proportion of the chicks which hatched were weak and died after a few days. Postmortem examinations of such chicks may reveal kidney lesions resembling those seen in embryos which fail to hatch. The liver of the embryo may also show a mottled appearance or whitish foci, but this is not a constant finding.

Activity of Field and Egg-Propagated Virus.—The results of the study of the incubation period and duration of the infection using the field and egg-propagated virus in mature and young birds is shown in table 3.

In the case of the field virus, the incubation period varied from seven to fourteen days, and the duration of infection from twenty-eight to forty-nine days. The symptoms shown by these birds were quite similar to those cases observed from the field outbreak, consisting of nasal exudation and respiratory râles.

Four mature birds which were inoculated with the egg-propagated virus manifested incubation periods varying between seven and fifteen days. The infection persisted in them for a period of twenty-four to twenty-eight days after the first symptoms were observed. The symptoms were similar to those resulting from inoculation with the field virus.

Three 6-week-old birds were inoculated with an egg-propagated virus, and 1 of the 3 developed symptoms after fourteen days which persisted over a period of about four weeks. The other 2 birds failed to show infection during the period of observation. The only symptom observed in this bird consisted of a nasal discharge.

Two groups of 1-week-old chicks were

TABLE 3—The Incubation Period and Duration of Infection in Mature and Young Birds Following Inoculation with Field and Egg-Propagated Virus

Type of Virus	Age of Bird	Routes of Inoculation			Incubation Period	Symptoms*	Duration of Infection
		Nasal Cavity	Trachea	Air Sac			
Field	Mature	+	+		7 days	R; NE	28 days
Field	Mature	+	+		14 days	R; NE	49 days
Egg Virus	Mature	+	+	+	7 days	R; NE	24 days
Egg Virus	Mature	+	+	+	7 days	R; NE	24 days
Egg Virus	Mature	+	+	+	14 days	R; NE	24 days
Egg Virus	Mature	+	+	+	15 days	R; NE	28 days
Egg Virus	6 weeks	+	+		14 days	NE	26 days
Egg Virus	6 weeks	+	+		None	None	.....
Egg Virus	6 weeks	+	+		None	None	.....
Egg Virus	1 week	+	+	+	6 days	NE	30 days
Egg Virus	1 week	+	+	+	15 days	NE	26 days
Egg Virus	1 week	+	+	+	17 days	NE	29 days
Egg Virus	1 week	+	+	+	7 days	NE	21 days
Egg Virus	1 week	+	+	+	8 days	NE	34 days
Egg Virus	1 week	+	+	+	10 days	NE	24 days
Egg Virus	1 week	+	+	+	12 days	NE	21 days
Chick Virus	2 weeks	+	+		5 days	NE	54 days
Chick Virus	2 weeks	+	+		8 days	NE	68†

*R = Râles; NE = Nasal exudate.
†This bird still showed the infection at the time the observations were discontinued.

inoculated with the egg-propagated virus and showed an incubation period varying from six to seventeen days. The duration of infection varied from twenty-one to thirty-four days, with termination in recovery. These chicks failed to show symptoms other than a coryza, even though they were inoculated intratracheally and into the air sacs.

This observation on susceptibility of baby chicks to laboratory exposure appears to be substantiated by the results seen under field conditions. The farm from which the infective material was originally obtained experienced the infection in 6,000 young chicks during January and February. The disease apparently did not affect the growth and was characterized only by symptoms of a coryza.

Two chicks inoculated with the exudate from the 2 latter experimental groups of birds showed incubation periods of five to eight days. The only symptoms consisted of a coryza which extended over a period of fifty-four and sixty-eight days.

The 7 birds which recovered from the infection with the egg-propagated material as chicks were inoculated at 9 weeks of age with infectious bronchitis virus. All were susceptible, thus indicating that immunity to the latter disease had not resulted from infection with the virus in question.

The reinoculation of the chicks with the specific virus failed to incite infection, indicating that immunity develops following recovery from the disease.

Gibbs[5,6] has reported the isolation of a virus causing a coryza of chickens, but since a description of the disease was not given, it is impossible to state whether any relationship exists between our virus and that which he described.

DISCUSSION

Investigations of a respiratory disease clinically indistinguishable from infectious bronchitis, but having a longer incubation period and longer course, resulted in the isolation of a virus which could be propagated in embryonating chicken eggs.

The growth of the virus in eggs was associated with death of approximately 25 to 50 per cent of the embryos after an incubation period of twenty-four hours, while the remainder of the embryos, in most instances, survived until hatching time.

The lesions of the embryos which died in twenty-four hours were characterized by hemorrhages over the skin and by marked congestion, enlargement, and hemorrhages of the kidneys. No lesions could be detected on the chorioallantoic membrane.

In those embryos surviving for several days, the lesions were characterized by whitish, circumscribed foci on the chorioallantoic membranes extending to areas beyond the points where the virus had been injected. The kidneys were frequently enlarged, congested, and showed retention of urates. The liver in some cases was mottled and enlarged, or showed whitish foci.

The general picture of the effects of the virus on the embryos did not appreciably differ from the fourth to the thirty-fifth passage, although its activity did seem to increase up to and including the fourth transfer.

The egg-propagated virus was capable of inciting infection in both mature birds and young chicks, after incubation periods varying from six or seven to seventeen days following inoculation. The duration of the apparent disease varied from twenty-one to sixty-eight days in individual birds. The incubation period was similar to that observed in birds which had been inoculated with the field virus. The disease in older birds was characterized by a coryza and respiratory râles, regardless of whether the field or the egg-propagated virus had been employed.

The disease induced by inoculation of young chicks was characterized only by a coryza which did not appear to be serious enough to affect growth. Similar symptoms were observed in infection among 6,000 young chicks from the farm where the virus was originally obtained.

Chickens which recovered from the virus infection were susceptible to the virus of infectious bronchitis, further demonstrating differences between the two diseases.

Recovered birds reinoculated with this virus failed to show any symptoms, thus

indicating that prior infection had stimulated development of a significant degree of immunity.

SUMMARY

A virus of a chronic respiratory infection of chickens was propagated in fertile chicken eggs through 35 passages. The egg-propagated virus was capable of reproducing typical symptoms in mature birds, as indicated by a coryza and respiratory râles. In young chicks, under laboratory conditions, the virus incited only a coryza. Birds immune to laryngotracheitis and infectious bronchitis were susceptible to the virus. Birds which recovered from the new virus resisted reinfection, but were susceptible to infectious bronchitis, thus indicating that there is no cross immunity between the diseases.

ACKNOWLEDGMENTS

The authors are indebted to Doctors Schmidt and Turk of the Division of Veterinary Science, Texas Agricultural Experiment Station, for valuable suggestions in the preparation of this manuscript.

ADDENDUM

After completion of this manuscript, another respiratory disease came to our attention, described by D. E. Stover (Stover, Donald E.: A Filtrable Virus, the cause of a Respiratory-Nervous Disorder of Chickens. *Am. J. Vet. Res., 3,* (1942): 207-213), which is characterized by symptoms involving both the nervous and respiratory tract. From the description given, we would not suspect any relationship between the two diseases.

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An Anatomical Study of Hermaphrodism in Goats

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HERMAPHRODITES occur in milk goats probably more frequently than in any other farm animal. It is believed by the author that failure to recognize hermaphrodism in its external manifestations is the answer to many inquiries as to why goats frequently fail to breed. Hence, does which are hermaphroditic are often kept for breeders. Cases have been overlooked even by veterinarians in inspecting goats for sale as breeding animals. Eaton and Simmons[7] reported on the occurrence of hermaphrodites over a period of fourteen years in the goat herd of the United States Department of Agriculture located at Beltsville, Md. In the Saanen breed, 11.1 per cent of the young were hermaphroditic while in the Toggenburgs there were 6 per cent. In this report, it was stated that from external appearance about half the hermaphrodites appeared to be males, the others females. Since then, a detailed study of the anatomy of the sexual organs has been made. The results are given in the present report.*

MATERIAL AND METHODS

Hermaphrodite kids from the Saanen and Toggenburg breeds were slaughtered in most cases at 10 days of age. Observations were made on the external genitalia before slaughter. After slaughter, the genital organs were carefully dissected out and preserved in 10 per cent formalin for future study. Sections were made of the various parts of the reproductive system from several cases for histological study. Comparison was made with normal male and female organs of goats of about the same age. In all, 29 cases were studied. A general description of each case is given in table 1.

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*Acknowledgment is due D. A. Spencer of the Office of Sheep and Goat Husbandry Investigations for making available the material for this study, and to Victor L. Simmons, who assisted in the dissection of the animals and furnished the birth data and ancestry of the cases studied.

TYPES RECOGNIZED

It is difficult to make an accurate classification of the types found, since there was considerable overlapping. However, an attempt was made to classify the types as to their external appearance, whether of the male or female type as determined by external genitalia, and as to internal structures present. Four sub-types were made for males and females, each according to general outward appearance, and 6 types were recognized in the internal structure. A brief description of the types as recognized externally follows:

A) Female type; external genitalia characteristically female in type or slightly modified from the normal female type. Penis and scrotum absent.

1) Normal female. The vulva projected slightly; the vulvar cleft began less than 1 cm. below the anal opening (in a 10-day-old specimen) and was about 1 cm. long. The clitoris was completely hidden by the lips of the vulva (fig. 1). Case 19.

2) Slightly bulbous vulva. The vulva projected slightly and in some, it had a swollen appearance. The vulvar cleft was represented by only a short slit or simply by a rounded opening. The clitoris was projected slightly (fig. 2). Cases 4, 10, 13.

3) Pronounced projecting bulbous vulva. This projected slightly downward or straight back for as much as 2.5 cm. or more. Usually, there was a slit in the dorsal surface which represented the vulvar cleft. This was only a blind passage with the urethral orifice at or near its anterior end (fig. 3). Cases 5, 6, 8, 12, 16, 17.

4) Projecting bulbous vulva with prominent projecting clitoris. The bulbous vulva was not projected as much as in type 3 above mentioned, but the clitoris projected prominently from the vulvar cleft and completely filled it. Resembled somewhat the glans penis of males. Usually, a slight suggestion of a raphe from the anal opening to the vulvar cleft was present (fig. 4). Cases 15, 18, 23, 26, 29.

B) Male type; raphe along some portion

of the penis, penis of some type present; scrotum and testes was or was not present.

1) Penile clitoris. Large projecting bulb with long prominent raphe, projection from bulb resembling glans penis with urethral process present. Urethral orifice about midway between anal opening and projecting glans. Case 1 was placed here rather than in the female group because a scrotum was present. This specimen was much older than the other cases observed, which may account for its difference in development (fig. 5). Case 1.

2) Shortened penis. A distinct penis, but varying in length from about 2.5 cm. to nearly normal. Usually terminated in a bulbous structure which did or did not have a urethral orifice. If the latter was absent, the bulb was swollen from the accumulation of urine and frequently broke open. A long raphe was usually present (fig. 6). Cases 11, 22, 24, 25.

3) Hypospadias. A penis with the ventral surface open for a part or a whole of its length, through which the urine seeped nearly constantly. Usually, a raphe anterior and another posterior to the open portion. Bulbous end of penis frequently present (fig. 7). Cases 7, 9, 21, 28.

4) Normal male. Penis complete and full length. Testes and scrotum usually present. In some of the cases examined, the penis was normal, but one or both testes were in the abdominal cavity (cryptorchid) (fig. 8). Cases 2, 3, 14, 20, 27.

This classification presented a series beginning with a normal female type and gradually grading into the normal male type.

In a similar manner internal genitalia may be classified into the following types from normal female to normal male.

1) Uterine horn with ovotestis. Only 1 case of this type was found. Externally, the specimen appeared as a normal female, and it was only through examination for another purpose that the condition present was found. Only the left horn of the uterus was present. The Fallopian tubes did not seem to be present, unless slight thickenings in the membrane somewhat anterior to a slight swelling, which proved on histological examination to be an ovotestis, may be considered as such. These were not definite

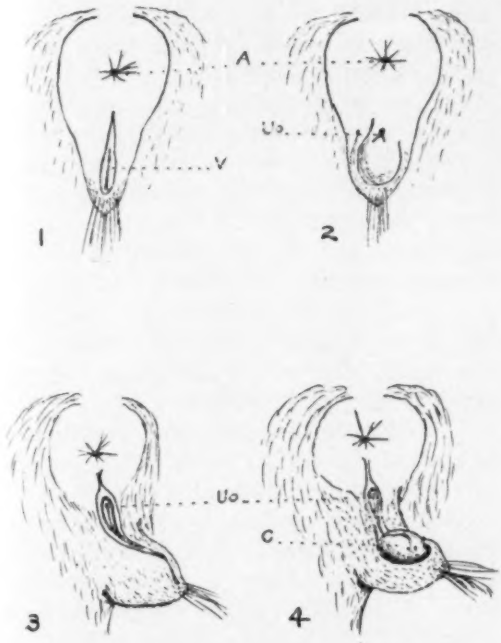
TABLE I—Summary of Structures Present in Hermaphrodite Kids*

Case No.	Age at Slaughter (days)	Bulbous vulva	Penis	Hypospadias	Long raphe	Scrotum	Testes	Seminal vesicles	Uterine horns	Vasa deferentia	Ovaries	Notes
1	93	+	—	—	+	+ +	+ +	+ +	+ +	+ +	— —	Scrotum small
2	440	—	+	—	—	+ +	+ +	+ +	— —	+ +	— —	Difficulty in unsheathing and erecting penis. Small scrotum
3	10	—	+	—	—	+ +	+ +	+ +	+ +	+ +	— —	Penis twisted directing urine rearward
4	10	+	—	—	—	— —	+ +	+ +	+ +	? ?	— —	Testes in abdominal cavity
5	10	+	—	—	—	— —	+ +	+ +	+ +	+ +	— —	Testes subcutaneous
6	11	+	—	—	—	— —	+ +	+ +	+ +	+ +	— —	Testes subcutaneous
7	10	—	+	+	+	— —	+ +	+ +	+ +	+ +	— —	Testes subcutaneous
8	13	+	—	—	—	— —	+ +	+ +	+ +	+ +	— —	Testes subcutaneous
9	10	—	+	+	+	— —	+ +	+ +	+ +	+ +	— —	Testes subcutaneous
10	9	+	—	—	—	— —	+ +	+ +	+ +	+ +	— —	Testes subcutaneous
11	10	—	+	—	—	— —	+ +	+ +	+ +	+ +	— —	Testes subcutaneous; penis twisted directing urine rearward
12	11	+	—	—	—	— —	+ +	+ +	+ +	+ +	†	Testes in abdominal cavity
13	10	+	—	—	—	— —	+ +	+ +	+ +	— —	— —	Testes in abdominal cavity
14	7	—	+	—	+	+ +	+ +	+ +	+ +	+ +	— —	Testes in abdominal cavity
15	‡	+	—	—	—	— —	+ +	+ +	+ +	+ +	— —	Right subcutaneous, left in abdominal cavity
16	10	+	—	—	+	— —	+ +	+ +	+ +	+ +	— —	Testes in abdominal cavity
17	11	+	—	—	+	— —	+ +	+ +	+ +	+ +	— —	Testes subcutaneous
18	9	+	—	—	—	— —	+ +	+ +	+ +	— —	— —	Testes in abdominal cavity
19	19	—	—	—	—	— —	— —	— —	+ —	— —	†	Right uterine horn absent
20	19	—	+	—	—	+ —	+ +	+ +	— —	+ +	— —	Monorchid
21	11	—	+	+	+	+ +	+ +	+ +	+ +	+ +	— —	
22	9	—	+	—	+	+ +	+ +	+ +	— —	+ +	— —	Cryptorchid
23	10	+	—	—	+	— —	+ +	+ +	+ +	? ?	— —	Testes subcutaneous
24	10	—	+	—	+	— —	+ +	+ +	+ +	+ +	— —	Testes subcutaneous
25	7	—	+	—	—	+ +	+ +	+ +	+ +	+ +	— —	Testes subcutaneous
26	11	+	—	—	—	— —	+ +	+ +	+ +	+ +	— —	Right subcutaneous, left abdominal
27	10	—	+	—	—	— +	+ +	+ +	— —	+ +	— —	Monorchid
28	10	—	+	+	+	+ —	+ +	+ +	? ?	+ +	— —	Monorchid
29	10	+	—	—	+	— —	+ +	+ +	+ +	+ +	— —	Testes subcutaneous

*+ Signifies presence of the structure, — its absence. + + or — — means its presence or absence on both sides. ? = Identity of structure not definitely determined. †Ovotestis. ‡Died soon after birth.

and lost themselves in the membrane (fig. 16). Case 19.

2) Uterus appeared nearly normal, but Fallopian tubes were absent, and instead of



Uo = urinary orifice; P = end of penis; H = hypospadias; S = scrotum.

Fig. 1 to 4—Female types of external genitalia. (1) Normal female, case 19. (2) Slightly bulbous vulva, case 10. (3) Pronounced projecting bulbous vulva, case 6. (4) Bulbous vulva with projecting clitoris, case 18.

ovaries, testes were present. Very small seminal vesicles were present (fig. 10). Cases 4, 13, 18.

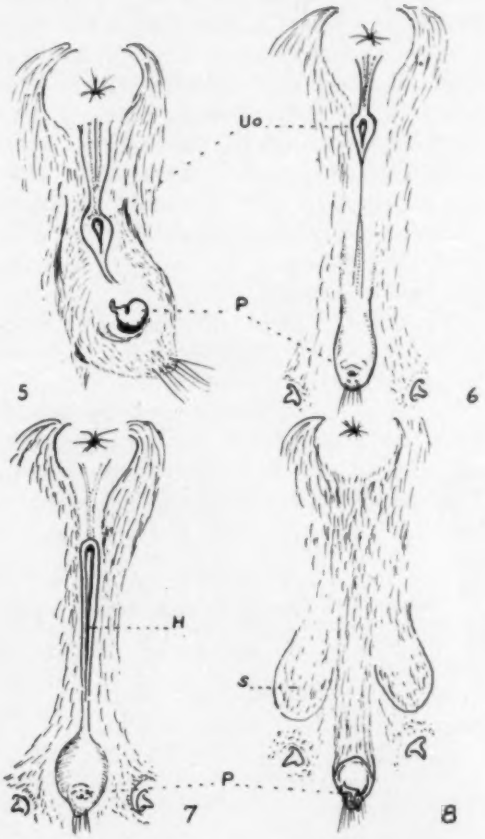
3) Uterus and small vasa deferentia were present. The latter, in some, were only a short remnant near the seminal vesicles, the anterior end of which disappeared in the membrane. Testes present either in body cavity or beneath skin of abdomen (fig. 11). Cases 23, 24, 29.

4) Uterine horns and vasa deferentia about equally developed. Same ran closely parallel throughout most of their length or were separated near their bifurcation. Seminal vesicles were present. Testes beneath skin of abdomen or in scrotum (fig. 12 and 13). Cases 5, 6, 7, 8, 9, 10, 12, 15, 16, 17, 21, 25, 26.

5) Uterine horns and vasa deferentia present. After they united, the former faded out in the membrane without extending as far as the union of the vasa deferentia. Testes were usually in scrotum (fig. 14). Cases 3, 11, 14.

6) Normal male. Vasa deferentia well developed; no remnant of uterus visible. Seminal vesicles much more developed than in previous cases. Testes were in scrotum or in body cavity. The latter were cryptorchids rather than hermaphrodites (fig. 15). Cases 2, 20, 22, 27, 28.

The relation between outer appearance and internal structures is shown in table 2.



F = Fallopian tube; G = gonad; O = ovary; U = uterus; B = urinary bladder; Sv = seminal vesicles; M = Müllerian duct; W = Wolffian duct.

Fig. 5 to 8—Male types of external genitalia. (5) Large bulb with penis-like projection, case 1. (6) Short penis with slightly bulbous end, case 21. (7) Hypospadias and bulbous process of the skin of the abdomen, case 7. (8) Normal male with penis and scrotum, case 3.

It will be seen that male and female appearing types as determined externally were nearly equal, agreeing with an earlier observation by Eaton and Simmons.[7] It

will also be seen that two of the types as determined by internal structure occurred only in those cases which presented a female outward appearance, and two other types occurred only in the male classification. The two remaining types were distributed between both males and females. Thus, there was a gradual transition from one type to the other so far as both external and internal development are concerned. This transition appears to be influenced by the type of structures present. When the normal female structures (Müllerian ducts) were more strongly developed, the animal appeared externally more like a female. If there was an approximately equal development of both male and female structures, some animals resembled either sex externally. If the male structures (Wolffian ducts) were more strongly developed, the external appearance of the animal was that of a male.

The description of a hypospadic Karakul lamb born at the Beltsville, Md., Research Center is given for comparison with these other cases. The lamb was born Feb. 27, 1940, and slaughtered the next day. This animal showed a characteristic hypospadias. The urethral opening was about 5 mm. below the anal opening. A long raphe extended for about 8 cm. along the abdominal wall and terminated in a bulbous process, oval in shape, just ahead of the scrotum. Testes were present in the scrotum. A single set of ducts (the Wolffian) was present, leading from the testes. Seminal vesicles were present and larger than those in the goat specimens. This animal closely resembled cases 7 and 9 of the goats except for the absence of Müllerian ducts in the lamb.

The cases observed were compared with a normal male and female kid of approximately the same age. The testes of a normal male kid 1 day old were located in a small pendant scrotum. Each testis was enclosed in a tough tunica vaginalis, and had leading from it to the abdominal cavity the spermatic cord which contained the vas deferens and blood vessels.

The testes in the cases studied resembled in general the normal testis. Those located in the scrotum or beneath the skin of the abdomen were enclosed in the tunica vaginalis, while those in the abdominal cavity lacked this structure. In some cases, the shape of the globus minor varied somewhat from the normal, being less bent. The normal testis at 1 day of age was larger than those of the 10-day-old hermaphrodites. The histological structure of the seminifer-

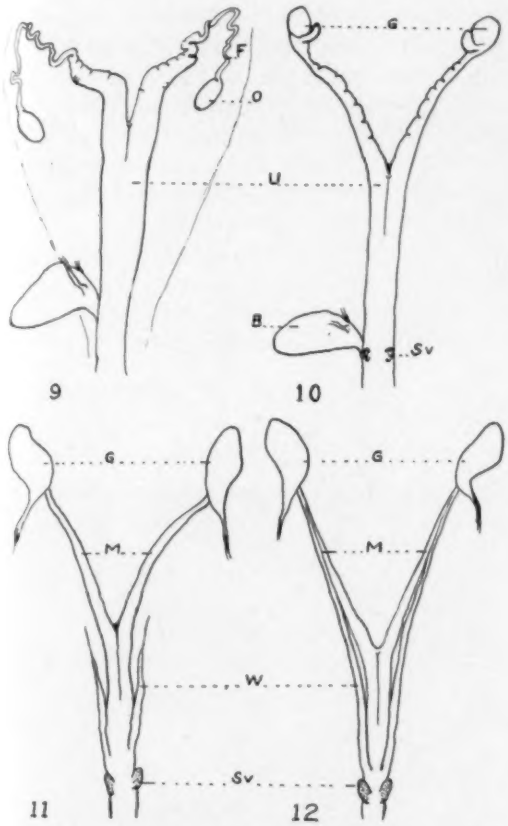
TABLE 2—Correlation Between External Appearance and Internal Structures in Hermaphrodite Kids*

		A) Female Type				B) Male Type				
Type	Internal Structures	Normal vulva and vagina	Small bulbous vulva	Large projecting bulbous vulva	Bulbous vulva with enlarged clitoris	Long raphe and bulbous penile clitoris	Short penis, no hypospadias	Hypospadias	Normal penis and scrotum	No. of cases
1	Uterus and ovotestis	19	..	..	..	..	..	..	..	1
2	Uterine horns and testes, small seminal vesicles	..	4,13	..	18	..	..	..	..	3
3	Uterus and small vas deferens and small seminal vesicles	..	..	..	23, 29	..	24	..	..	3
4	Uterus and vas deferens, small seminal vesicles	..	10	5, 6, 8, 12, 16, 17	15, 26	1	25	7, 9, 21	..	14
5	Incomplete uterus, large seminal vesicles	..	..	..	..	..	11	..	3, 14	3
6	Normal vas deferens and seminal vesicles, uterus absent	..	..	..	..	..	22	28	2, 20, 27	5
Total		1	3	6	5	1	4	4	5	29
Total		15				14				29

*Numbers in each square signify case numbers.

ous tubules, efferent tubules, vas epididymis, and vas deferens did not differ from those of the normal specimen. Probably, no difference should be expected at this age. In the two specimens 93 and 440 days of age, the seminiferous tubules were somewhat shrunken, possibly a result of the fixation process. No spermatogensis was observed in the 440-day-old specimen.

In nearly every case examined, seminal vesicles were present. They varied in size from nearly normal (5 by 8 mm. in the normal 1-day-old specimen) to less than half this size.



G = gonad; M = Müllerian duct; W = Wolffian duct; Sv = seminal vesicles; T = testis; Ot = ovotestis; Vd = vas deferens; U = Uterus.

Fig. 9 to 12—Diagram of types of internal genitalia. (9) Normal female type with ovaries, oviducts and uterus. (10) Nearly normal type of uterus, but with testes present and very small seminal vesicles, case 4. (11) Uterine horns and remnants of the Wolffian ducts, small seminal vesicles, case 23. (12) Both Müllerian and Wolffian ducts present and closely parallel, case 6.

The structure found in case 19 was interpreted as an ovotestis. This was about 2 by 4 mm. and appeared to be located in the uterine ligament which supported the left uterine horn. The right uterine horn was absent. Leading from the ovotestis craniad were two tube-like structures which became lost in the membrane. Sections of the ovotestis (fig. 19) showed that about one half resembled the rete testis with its irregular canals. The other half resembled ovarian tissue. Near the surface was a single primitive Graafian follicle. This was oval in form and surrounded by epithelial cells. The dark staining nucleus and darker nucleolus occupied the center of the ovum. At least 3 similar primary follicles were found in the right testis of case 12. Here

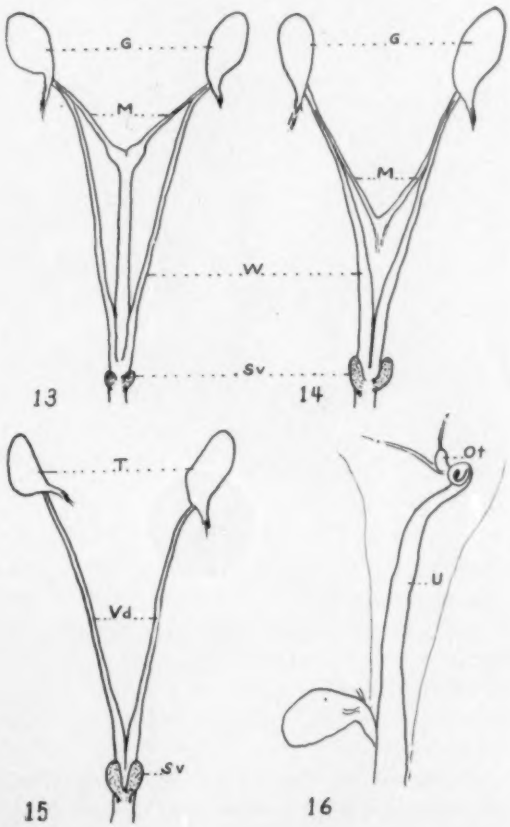


Fig. 13 to 16—Diagram of types of internal genitalia. (13) Müllerian and Wolffian ducts separated, case 7. (14) Wolffian ducts and seminal vesicles well developed, lower portion of Müllerian ducts degenerating, case 3. (15) Normal male with vasa deferentia and seminal vesicles, case 2. (16) Unicorn uterus and ovotestis of case 19.

they were located in the connective tissue around the outside of the testis near its caudal end (fig. 21). The left testis was not sectioned. These follicles resembled the structure photographed by Witschi and Mengert[17] in a human hermaphrodite, except that the goat follicles were not located in cystic cavities.

Similar structures were not found in the other testes sectioned. In some cases, the testis was not sectioned in a location which would show the follicles had they been present.

In most of the cases examined, there were present both male and female structures, designated in table 1 as vasa deferentia and uterine horns. These are derived from embryonic structures known as Wolffian and Müllerian ducts, respectively. In the cases examined, complete sexual differentiation had not taken place and both sets of ducts persisted in whole or in part, whereas, in a normal male only the Wolffian persist, and in the female the Müllerian. The other duct system in each case degenerates except for a few vestiges found in the epididymis or in the ovary. In several cases it was difficult to determine if one or both duct systems were present. In several cases, the identity of the parts was determined by their histological structure. In some other cases where sections were not made, there still remained a question as to the real identity of the structure present. In most cases, however, the relationship of parts aided in their identification.

TABLE 3—Parentage of the Cases of Hermaphrodism Included in the Anatomical Study

Case No.	Toggenburg Sire	Toggenburg Dam	Saanen Sire	Saanen Dam	Notes regarding parturition
1	A	954	..	...	Twin to normal buck
2	..	...	E	664	One of triplets, 1 buck, 1 hermaphrodite
3	A	954	..	...	One of triplets, 1 buck, 1 doe
4	..	...	F	869	One of triplets, 2 normal does
5	A	970	..	...	Twin to normal buck
6	..	...	F	859	Twin to normal buck
7	..	...	F	105	Twin to normal buck
8	..	...	F	107	Single
9	..	...	F	857	One of triplets, 2 normal bucks
10	..	...	F	466	Twin to normal buck
11	..	...	E	867	One of triplets, 1 buck, 1 doe
12	..	...	G	869	Twin to normal doe
13	..	...	G	128	Single
14	..	...	G	857	Triplet with 2 normal does
15	B	774	..	...	Twin to normal buck
16	..	...	G	867	Twin to normal buck
17	C	22	..	...	Twin to normal buck
18	C	933	..	...	Twin to normal buck
19	C	717	..	...	One of triplets, 1 buck, 1 hermaphrodite
20	C	717	..	...	One of triplets, 1 buck, 1 hermaphrodite
21	..	...	H	126	Twin to normal buck
22	B	774	..	...	Single
23	B	68	..	...	Twin to doe
24	B	51	..	...	One of triplets, 1 buck, 1 doe
25	B	941	..	...	Single
26	A	83	..	...	Twin to buck
27	..	...	H	171	Twin to doe
28	D	67	..	...	Single
29	..	...	F	857	Single

A = anal opening; Uo = urinary orifice; V = lips of vulva; C = clitoris.

HEREDITY OF HERMAPHRODISM

The parentage of the cases studied is shown in table 3. It will be noticed that bucks A, B, and C sired all but one of the 14 cases in the Toggenburg breed and bucks F and G most of the cases in the Saanen breed. In three cases among the Toggenburgs and two among the Saanens, a single dam produced two specimens. In the Saanens, 1 other doe also produced 3

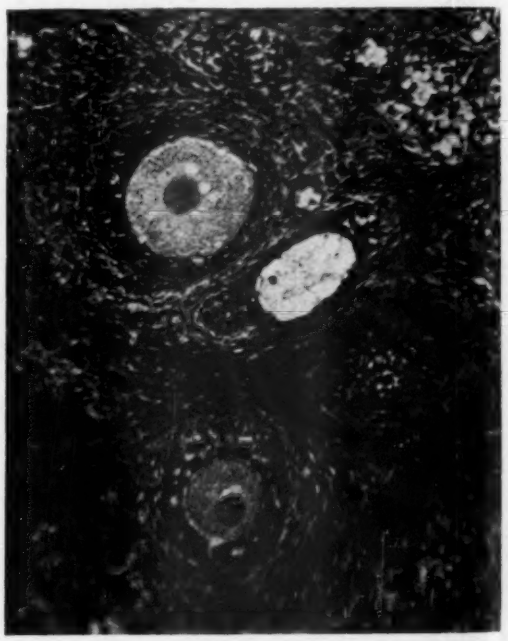


Fig. 17—Ovary of normal female kid 9 days old. x150.

of the cases listed. In the Saanens, bucks F, G, and H were son, grandson, and great-grandson, respectively, of buck E. There were 8 other bucks in the herd, all of which sired at least 1 hermaphrodite kid. There were 25 does which produced 95 normal kids and no hermaphrodites when mated to these bucks. Twenty other does mated to these bucks produced 102 normal and 32 hermaphrodite kids.

Among the Toggenburgs, bucks B and D were son and grandson, respectively, of buck A. Buck C was also a grandson of A, but from a different sire than D. The sire of C also sired hermaphroditic kids, none of which, however, were in this anatomical study. There were 12 bucks in the herd besides the ones named. Three of these, when mated with 17 does, produced 28 normal kids and no hermaphrodites. Another buck sired only normal young with various does, but some of the does when mated with other bucks, gave birth to hermaphrodite kids. Six does which produced hermaphrodites with other bucks, gave only normal young when mated with 3 of these 4 apparently free bucks. The other 8 bucks produced 74 normal and 21 hermaphrodite kids when mated with 17 does. These results from both breeds closely approach a 3 : 1 ratio when animals which produce hermaphrodites were mated together. This suggests that a single recessive gene was involved, and that it was transmitted only by heterozygous individuals, since the recessive homozygote was incapable of reproduction.

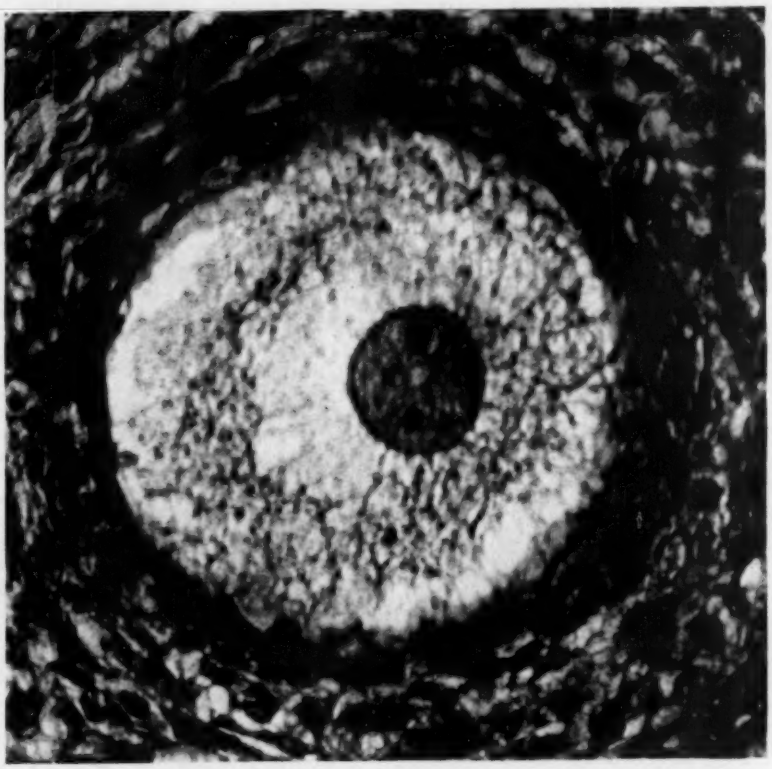


Fig. 18 — Enlargement of follicle from ovary of normal kid 9 days old. x600.

It will also be noticed that the presence of a hermaphrodite kid in cases of multiple births apparently did not affect the normal sexual character of the other kid or kids born during the same parturition, since these others may have been normal bucks or does. This differs from the case of the free-martin in cattle where the female cotwin to a bull calf is sterile. This is true, however, only if there is an anastomosis of the fetal blood vessels.

DISCUSSION

In order to understand the development and differentiation of sex, a brief discussion of early embryology is necessary. Sex is determined genetically by the chromosome combination, in mammals the XX combination being female and the XY combination being male. For a certain period, the developing embryo is morphologically indifferent sexually. During this indifferent period, a system of ducts, the Wolffian and Müllerian, are laid down. The former appear first and develop equally in both sexes until sexual differentiation begins, when they finally become the canals of the epididymis and the vas deferens in the male and degenerate in the female except for vestigial structures near each end. The Müllerian ducts arise later and upon sexual differentiation form Fallopian tubes, uterus, and vagina in the female and degenerate in the male except for terminal vestigial structures. During sexual differentiation, several changes take place which alter the form and character of the cells which form the sex organs. In the chick, calf, pig, and rabbit, the interstitial cells which are considered the main source of the sex hormones appear early in the testis, but not until a considerably later period in the ovary. In the calf,[2] whose embryology probably corresponds more closely to that of the goat, the interstitial cells appear in the testis in the 2.7- to 3.0- cm.-stage embryo, while in the ovary they do not appear until the 82.0-cm. stage (near birth). During the sexually indifferent period, the urinogenital sinus forms a common opening for the Müllerian, Wolffian, and metanephric ducts (later ureters). The urinogenital sinus later forms the urethra. Near the caudal end of the Wolffian ducts, saclike evaginations arise and form the seminal vesicles of the male. A genital tubercle arises in the midventral line of the body wall in front of the anal pit and forms the penis of the male or the clitoris of the female.

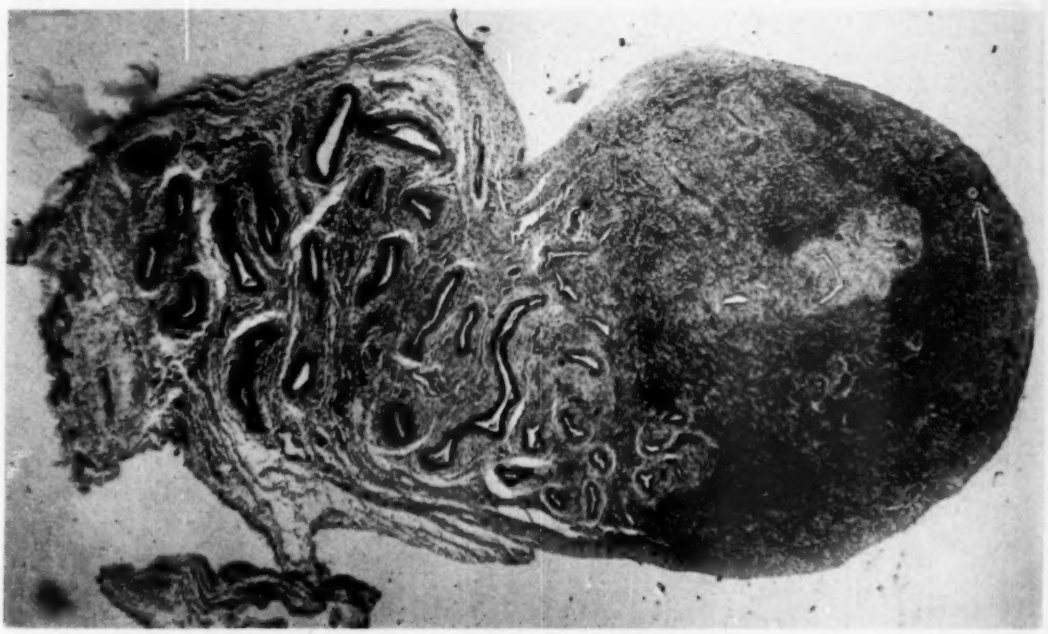


Fig. 19—Section of ovotestis of case 19. x28.6. Position of follicle indicated by arrow.

The sex hormones do not cause new structures to develop, but stimulate or suppress the development of parts already laid down. The genetic constitution of the individual determines which system of ducts shall develop normally. However, some factor may

intervene and upset the normal course of development. Goldschmidt,[9] Crew,[5] and Brambell[3,4] propose a time relation factor.

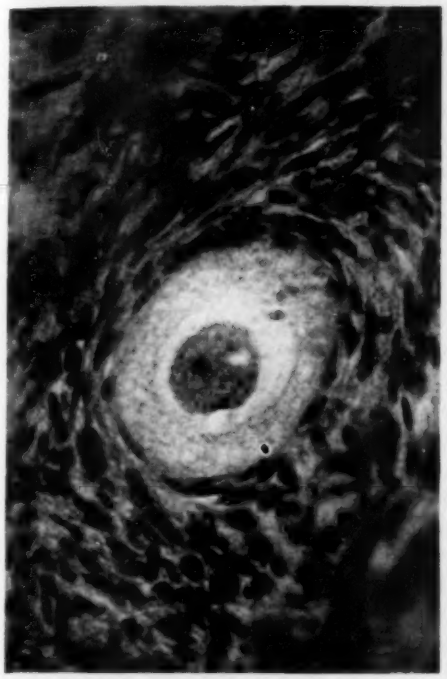


Fig. 20—Enlargement of follicle from ovotestis of case 19. x600.

There may be a retardation in the development of the interstitial cells, causing a delay in the elaboration of their hormonal substance, or elaboration of the hormones may proceed at different rates. Thus, in the absence of proper endocrine control, both systems of ducts will develop parallel and an overlapping of the structures of both sexes will result. The amount of overlapping will depend on the time of onset of hormone action, quantity of hormone below the necessary threshold for normal development, and duration of the period of insufficient hormone action. Thus, various grades of intersexuality will appear.

The first to confirm this hormone relation to sex development was Lillie.[14] Since then, many experiments have further confirmed and aided in a better understanding of sexual differentiation.

The experiments of Hamilton and Wolfe,[12] Greene and Burrill,[10] Greene, Burrill and Ivy,[11] Turner,[16] and others have shown that when female rats are injected late in pregnancy, the female young are masculinized in appearance, having large clitoris, testes, vasa deferentia running parallel with the uterus, and frequently hypospadias. The

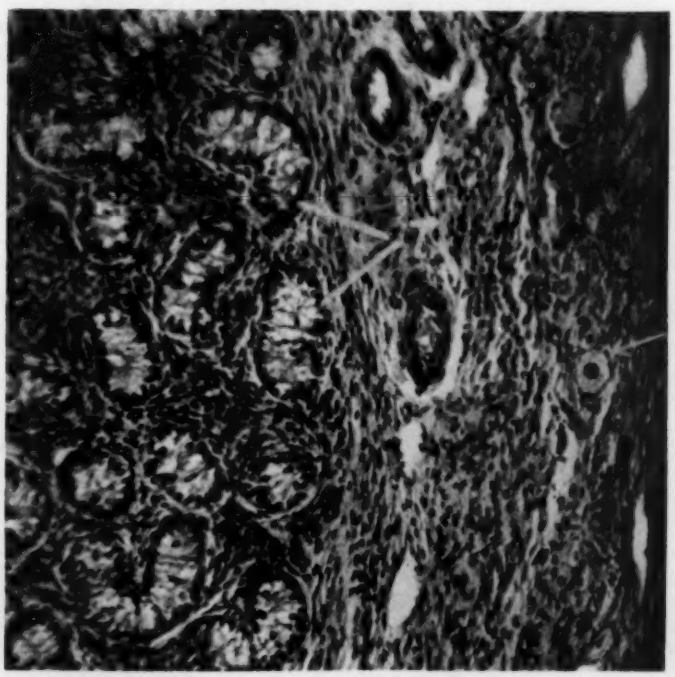


Fig. 21—Ovotestis of case 12 showing seminiferous tubules and follicle in cortex. Arrow indicates position of follicle; T; seminiferous tubules. x150.

results are apparently the same whether male or female hormones are used. Males are unaffected. The same external appearance may also be obtained by injecting the young up to about seven days after birth. Thus, it appears to be the distal portion of the female ducts and the urogenital sinus, the parts last to differentiate, that are affected most by hormone injections. Since the male organs are differentiated earlier, they are unaffected. The degree of masculinization depended upon the amount of substance used and on the period of pregnancy when treatment was given.

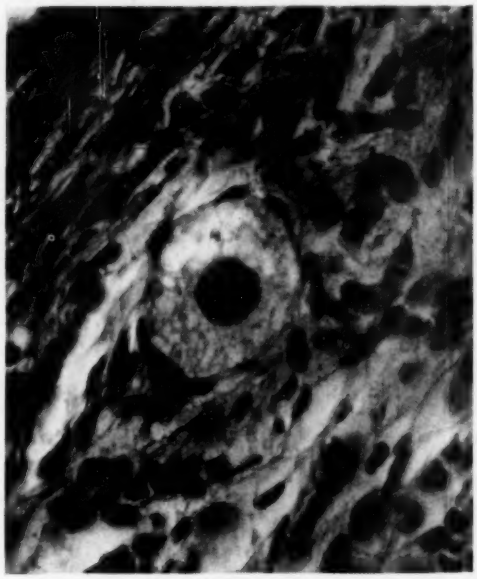


Fig. 22—Enlargement of follicle from case 12. x600.

The appearance of the goat hermaphrodites agreed in many instances with the conditions produced experimentally in rats. Case 2 probably should not be classed as a hermaphrodite. The testes were not fully pendant in the scrotum and the spermatic tubules were not normal in structure, suggesting more the cryptorchid condition. Some minor abnormality may have accounted for the inability to unsheath and erect the penis.

Crew and Dighton[6] reported that the structure of the testes varied according to their position. In the present data, so far as could be observed, the structure of the testes was the same regardless of their position, except that those located within the abdominal cavity lacked the tunica vaginalis. In other respects, the present data agree very well with those of the reference in question. Had the kids been older, undoubtedly there would have been demonstrable histological differences.

It appears that the hypospadias is related to hermaphrodism since 3 of the 4 cases presented showed the presence of both Müllerian and Wolffian ducts. The hypospadias in 2 of these cases was very slight, in case 21 being a slit about 6.0 mm. long located 2.2 cm. below the anal opening, and in case 28 there was actually no open split, but a loose pouch of skin about 3.0 cm. long, near the end of which was a small opening representing the phallic portion of the urethra.

The 2 cases of ovotestis found confirm Asdell's[1] statement regarding their rarity, of which he reports only 2 cases among many examined. Asdell states also that the type of hermaphrodism he observed in England differs from that in America, the former being of the female type and the latter of the male type. The data presented here consist of both types in nearly equal numbers and the two types agree fairly well with those of Asdell so far as can be determined from his descriptions. Krediet[13] reported a case in a mare of an ovotestis which showed a single follicle like case 19, but differed from it by the presence of certain male structures. Fincher and Williams[8] reported on 3 calves in which the right uterine horn was absent.

Cases 20, 27, and 28 should probably be classed as cryptorchids, although other cases in which one or both testes were in the abdominal cavity and both male and female structures were present suggest that there may be a relation between hermaphrodism and cryptorchidism. Lush, Jones, and Dameron[15] reported the occurrence of cryptorchidism with quite high frequency among Angora goats. The question arises whether in their material there might have been some of the female structures present.

CONCLUSION

The data presented and the comparison with other reported cases of hermaphrodism seem to indicate that hermaphrodism, hypospadias, and cryptorchidism are closely allied, probably all traceable to abnormal hormonal conditions during the time of sexual differentiation.

Evidence also points strongly to a hereditary basis for these abnormalities. Heredity is probably in the nature of a gene regulating the development and activity at the proper time of the cells which are responsible for hormone secretions. The dominant gene controls normal development, its recessive allelomorph causes the disturbances which result in the abnormal conditions described.

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Chemotherapy of Streptococcal Bovine Mastitis

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IT IS GENERALLY considered that mastitis is one of the most widespread and costly diseases now confronting the dairyman. Relatively few dairy herds have been found free of streptococcal mastitis, and records indicate that approximately 20 to 50 per cent of the cows in the average herd are infected. Tissue injury due to this infection reduces the productive capacity of the animal and the amount of saleable milk and is one of the major causes of the rapid turnover of cows in commercial dairy herds. Several investigators have estimated that the milk production of quarters infected with *Streptococcus agalactiae* is about 25 per cent less than that of normal quarters. In one of the experimental herds here reported, records on 14 quarters infected with streptococci showed a decrease of 51.2 per cent in production when compared with the yield of corresponding noninfected quarters of the same animal.

Until recent years, most of the work on the chemotherapy of bovine mastitis had been done in Europe. For nearly two decades, workers abroad have reported on the efficacy of intramammary infusions with various acridine dyes in the treatment of streptococcal mastitis. Perhaps, the most extensive work in this respect has been done by Steck[1-6] in Switzerland. In this country, both Schalm[7-9] and Johnson[10] have found acridine therapy effective in freeing quarters of streptococcal infections. In the original work by Little and associates,[11] an aqueous solution of gramicidin infused into the quarter was found to exhibit a definite bactericidal effect against udder streptococci. In his later work,[12-13] temporary emulsions of this agent in mineral oil were employed with more favorable results. Since that time, tyrothricin has been used by several workers[9, 14-16] in the treatment of streptococcal mastitis. Tyrothricin is a bacterial extract obtained from *Bacillus brevis*, containing both gramicidin and tyrocidin, first isolated by Dubos[17] in 1939. Klein *et al.*[18] and others[9, 19, 20] found colloidal silver oxide (Novoxil) effective in eliminating *Str. agalactiae* from a high percentage of infected quarters. Of interest is a recent report by Sanders[21] on the use of iodized mineral oil as a treatment for bovine mastitis. It appears from his work that iodine has a marked bactericidal effect on mastitis-producing microorganisms.

From the literature cited, it appears that treatment of streptococcal bovine mastitis with these chemotherapeutic agents is of value in certain cases and selected herds. However, whether such treatment will prove to be of practical use under field conditions remains to be determined. In general, to be of practical use such an agent should eliminate a high percentage of infections with relatively few applications, cause little, if any, tissue damage or changes in the milk, and be comparatively inexpensive and easily administered.

In an attempt to determine the value of chemotherapy in controlling mastitis, combined with recognized sanitary and herd management procedures, an investigation has been undertaken for the treatment of a large number of infected animals in commercial dairy herds. A report of the results obtained in the first year is presented herein.

EXPERIMENTAL HERDS

Six commercial dairy herds, comprising 485 milking cows, located within a 30-mile

Presented before the Section on General Practice at the seventy-ninth annual meeting of the American Veterinary Medical Association, Chicago, Aug. 24-27, 1942.

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radius, have been included in the investigation up to this time. Selection of these herds was based on a reasonably high incidence of streptococcal mastitis as disclosed by a preliminary examination, the expressed willingness of the owner to give the required coöperation, and facilities adequate to carry on the work effectively. For convenience, these herds have been designated as A, B, C, D, E, and F.

Herd A was composed of 121 milking animals, with about an equal number of purebred Ayrshires, Guernseys, Holstein-Friesians, and Jerseys, and had a history of chronic mastitis of several years standing. Earlier attempts to control the disease through the use of the strip cup, brom-thymol-blue test, and infrequent laboratory examinations had failed. The pretreatment examination in this herd disclosed 115 quarters infected with *Str. agalactiae* in 43 (35.5%) of the animals. Twenty-eight of these quarters showed visible changes in the milk by the strip cup examination and 56 were positive to the brom-thymol-blue test. Physical examinations of 103 of these quarters revealed 31 to be slightly indurated, 65 distinctly indurated, and 7 markedly indurated. Six cows in the herd were shedding *Streptococcus uberis* regularly in the milk from 10 quarters, and *Streptococcus dysgalactiae* was present in a single quarter of another cow. Several of these latter quarters had definite symptoms of mastitis and produced abnormal milk. Fourteen (11.6%) of the cows showed *Staphylococcus aureus* on the initial examination. After the initial test, the animals were segregated into noninfected and infected groups in the milking barn. Separate milking machines were assigned to each group and additional sanitary measures instituted. Cows undergoing treatment were moved to another barn and milked by hand.

In herd B, there were 66 grade cows, mostly Holstein-Friesians. Of these, 22 (33.3%) were harboring *Str. agalactiae* infection in 1 or more quarters. At the time of the initial examination, acute symptoms were found in 12 quarters of 6 cows. Fifty-three of 59 quarters infected with *Str. agalactiae* showed alkaline reactions to the brom-thymol-blue indicator. The secretion from 35 contained flakes, blood, or pus, or was watery in appearance. Physical examination of 50 quarters showed 11 to be slightly indurated, 26 distinctly indurated, and 13 markedly indurated. Five quarters could not be palpated because of extensive edema. *Str. uberis* and certain unidentified types of streptococci were found in a number of cows. In most instances, these infections appeared sporadically and usually could not be found on the next examination. One quarter became infected with *Str. uberis* after a teat injury and four months later was still shedding this organism in the milk. *Sta. aureus* was present in 27 (40.9%) of the 66 cows on the initial examination. The animals infected with *Str. agalactiae* were moved to an adjoining barn for treatment and were hand milked. In addition, certain sanitary procedures were instituted.

Herd C.—There were 150 milking cows in this herd, with about equal numbers of grade and purebred Guernseys. A severe outbreak of clinical mastitis and a marked drop in the milk production had occurred in the herd just prior to the initial examination. At this time, 55 (36.6%) of the cows were found to be infected with *Str. agalactiae* and several were still showing clinical symptoms. A number of the animals were carrying infections of *Str. uberis*, *Streptococcus fecalis*, and a few unidentified streptococci. Of 159 infected quarters, 44 were producing abnormal milk as determined by the strip cup, and 67 were positive to the brom-thymol-blue test. No fibrosis could be detected in 3 quarters on physical examination, 17 were slightly indurated, 54 distinctly indurated, and 12 markedly indurated. Seventy-three quarters were not examined. The incidence of *Sta. aureus* in this herd was 16 per cent on the initial examination. Since the barn was so constructed as to provide adequate facilities for complete segregation and separate handling of the infected animals during milking, it was decided to treat the cows during the dry period.

Herd D was made up of 45 grade Guernseys and Holstein-Friesians. This herd also had a history of a number of cases of acute mastitis before the first examination. Four of these cases had terminated fatally. The survey preceding treatment revealed 6 (13.3%) of the animals to be infected with *Str. agalactiae* in 13 quarters, and *Str. uberis* in 13 quarters of 8 animals. Three quarters infected with *Str. agalactiae* were positive to the brom-thymol-blue test and 2 were producing abnormal milk. Four quarters infected with *Str. uberis* were producing alkaline milk. The milk of 3 of these quarters was watery in appearance. At this time, palpation of the infected quarters showed 10 slightly indurated, 8 distinctly indurated, and 7 markedly indurated. One quarter was not palpated because of extensive edema accompanying recent calving. Twenty-seven (60%) of the cows were infected with *Sta. aureus* in 1 or more quarters on the initial examination.

Herd E.—Forty-nine mature cows, mostly Holstein-Friesians, composed this herd. Of these, 23 (46.9%) were infected with *Str.*

agalactiae in 45 quarters. Six cows were infected in 6 quarters with *Str. uberis*. A number of these animals were producing abnormal milk and were milked by hand. Cows producing apparently normal milk were milked by machine. Of the 51 quarters harboring streptococcal infections, 26 were producing alkaline milk and 23 showed visible evidence of mastitis on the strip cup. Palpation disclosed 18 quarters to be slightly indurated, 21 distinctly indurated, and 9 markedly indurated. Three quarters showed marked edema. The initial examination revealed 4 cows (8.2%) with *Sta. aureus* infection.

Herd F, a herd of 54 Guernseys, Holstein-Friesians, and Jerseys, was shedding *Str. agalactiae* in the milk from 45 quarters of 17 (31.5%) of the animals. Eleven quarters of 4 cows were found to be infected with *Str. uberis* and atypical streptococci. Fifteen of 46 quarters examined with the strip cup produced abnormal milk, and 21 were positive to the brom-thymol-blue test. Physical examination of these quarters showed 9 slightly indurated, 10 distinctly indurated, 13 markedly indurated, and 14 edematous. Seven (13%) of the cows were infected with *Sta. aureus*. The diseased animals were segregated at the end of the line in the dairy barn and milked last by hand.

TABLE I—Incidence of Streptococcal Infection

Herd	Total No. of Cows	*Streptococcus agalactiae* Cows (No.)	Cows (%)	Quarters (No.)	*Streptococcus uberis* Cows (No.)	Cows (%)	Quarters (No.)	Other Streptococci Cows (No.)	Cows (%)	Quarters (No.)
A	121	43	35.5	115	6	4.9	10	4	3.3	4
B	66	22	33.3	59	2	3.0	2	2	3.0	2
C	150	55	36.6	117	7	4.6	8	20	13.3	34
D	45	6	13.3	13	8	17.9	13	0	...	0
E	49	23	46.9	45	6	12.2	6	1	2.0	3
F	54	17	31.5	45	3	5.6	9	1	1.9	2
Total	485	166	34.2	394	32	6.6	48	28*	5.8	45*

*Six quarters of 5 cows harboring *Streptococcus dysgalactiae* and *Streptococcus fecalis* included in these totals.

Experimental Procedure

Pretreatment Examination. — The preliminary examination of each animal consisted of the use of the strip cup and brom-thymol-blue test on each lactating quarter and collection of a composite sample of foremilk for bacteriological studies. The physical appearance of the milk and reactions to the brom-thymol-blue test were recorded at the farm. Within a few hours, the aseptically drawn milk samples were brought to the laboratory. Part of the sample was used for the Hotis[22] test and the remainder incubated over night for microscopic examination. The reactions to the Hotis test were read after twenty to twenty-four hours incubation at 37 C.

On the second examination, the strip cup and brom-thymol-blue test were used on each quarter. To determine the extent of the infection, a foremilk sample was taken from each quarter of all cows showing evidence of mastitis to any of the tests or in which streptococci were found bacteriologically. Composite samples were also collected from the negative cows. Each sample was examined by means of the Hotis test and microscopically.

All milk samples positive or suspected of containing mastitis-producing bacteria, as determined by the two foregoing tests, were cultured on 5 per cent bovine blood agar. After incubation, the plates were examined and colonies resembling streptococci or staphylococci were inoculated into 10 per cent horse-serum bouillon. For the purpose of identifying the streptococci biochemically, 0.1 cc. of each culture was inoculated into the usual differential mediums. Cultures of staphylococci were tested on litmus milk, methylene blue milk, lactose, salicin, mannite, and aesculin. The coagulase test, using rabbit blood plasma, was run on most of these cultures.

Before treatment, the milked out udder was examined physically and the quarters classified according to the system outlined by Udall.[23]

Treatment.—Just prior to treatment, the cow's udder and right flank were washed with a suitable strength of chlorine solu-

tion or other dairy disinfectant and dried with a paper towel. The ends of the teats were disinfected with a pledget of cotton moistened with a 1 to 1,000 solution of bichloride of mercury in an equal part of 95 per cent alcohol.

For administration of acriflavine and Entozon* solutions, a graduated 800-cc. infusion bottle with rubber tubing and a stainless steel teat tube was used. The apparatus and freshly prepared solutions were sterilized in the autoclave a few hours prior to treatment. The methods used in infusing quarters with acridine dyes were essentially the same as those described by Schalm.[7-8] Solutions of acriflavine were made up in 1 to 4,000 and 1 to 1,500 concentrations with both distilled water and 20 per cent sucrose solution. Entozon solution was made in a concentration of 1 to 1,250 with distilled water. All of the milk was withdrawn from the quarter before treatment. A preliminary rinse was used in approximately half the quarters and dispensed with in the others. The principal infusion varied from 400 to 1,600 cc., depending upon the size of the quarter. Acriflavine was left in the lactating quarter five to seven minutes, usually, and never over ten minutes. Entozon was left in the quarter thirty to sixty minutes. Treatment was repeated at from 7- to 21-day intervals. Cows treated during the dry period received 1 infusion which was left in the quarter over night and milked out the next morning. In a few instances, the solution was left in until the cow freshened.

For the injection of colloidal silver oxide and tyrothricin,† 10- and 50-cc. glass syringes, 18-gauge hypodermic needles, and Bowen‡ teat tubes attached to a short piece of rubber tubing were used. This equipment was sterilized in the autoclave before use.

Colloidal silver oxide was injected into lactating animals after the udder was completely milked out and left in the quarter until the next milking. In most instances, 10-cc. amounts of a 5 per cent suspension were injected. Doses of 13, 14, and 15 cc. were used in a few quarters. The interval between treatments varied from one to twenty-six days. In 22 lactating quarters, the recommended treatment of 3 injections at 24-hour intervals was used. Dry quarters were given 1 injection which remained in the udder until the animal freshened. The interim between treatment and drying-off varied from 18 to 161 days and between treatment and freshening, from 2 to 116 days.

Following the technique used by Little,[12] tyrothricin emulsion was prepared by diluting the alcoholic solution containing 40 mg. of the agent per cc. with 15 cc. of sterile distilled water and 25 to 35 cc. of sterile mineral oil. Immediately before injection, the mixture was thoroughly emulsified by shaking vigorously and drawing back and forth in the syringe several times. The dose of the bactericidal agent varied from 40 to 120 mg. and was left in the lactating quarter until the next milking. The interval between treatments was from four to seven days. Cows treated dry received 1 treatment from 8 to 123 days after drying-off, and from 2 to 116 days before freshening. The material was left in the udder until the cow calved.

The 4 chemotherapeutic agents were used on 156 lactating and 152 dry quarters. Acriflavine was used on 28 lactating and 7 dry quarters, Entozon on 30 lactating and 11 dry quarters, colloidal silver oxide on 63 lactating and 83 dry quarters, and tyrothricin on 35 lactating and 51 dry quarters. Except in a few instances, each quarter was treated no more than three times, as additional treatment was considered uneconomical under field conditions.

Post-Treatment Examinations.—Milk samples were collected within three days after treatment and at regular intervals thereafter. The results of treatment were not finally determined until two or more bacteriological examinations had been made after an interval of three weeks. During this time, several brom-thymol-blue tests, strip cup, and physical examinations were made on the quarters.

*Supplied by Winthrop Chemical Company, Inc.

†Supplied by Merck & Company, Inc.

‡Manufactured by Becton, Dickinson & Company.

RESULTS

The results of the bacteriological examination of foremilk samples from 485 cows appear in table 1. This examination disclosed 166 (34.2%) of the animals infected with *Str. agalactiae* in 394 quarters and 32 (6.6%) with *Str. uberis* in 48 quarters. One cow was shedding *Str. dysgalactiae* in the milk of 1 quarter, and *Str. fecalis* was found in 5 quarters of 4 other cows. Infections with unidentified streptococci were found in 39 quarters of 23 cows.

TABLE 2—Efficiency of Chemotherapeutic Agents

Agent	Lactating Quarters			Dry Quarters		
	No. Treated	Freed of Infection (No.)	(%)	No. Treated	Freed of Infection (No.)	(%)
Acriflavine	28	25	89.3	7	5	71.4
Entozon	30	18	60.0	11	8	72.7
Colloidal silver oxide	63	45	71.4	83	58	69.9
Tyrothricin	35	18	51.4	51	47	92.1
Total	156	106	67.9	152	118	77.6

Sta. aureus infection was present in 103 (21.2%) of the animals on the initial examination.

As shown in table 2, 106 (67.9%) of the lactating quarters responded to treatment and 118 (77.6%) of the quarters treated during the dry period were found free of streptococcal infection after freshening. In the lactating quarters, acriflavine was found to have an efficiency of 89.3 per cent in destroying streptococcal infections, Entozon 60 per cent, colloidal silver oxide 71.4 per cent, and tyrothricin 51.4 per cent. In dry quarters, the efficiency of acriflavine was 71.4 per cent, Entozon 72.7 per cent, colloidal silver oxide 69.9 per cent, and tyrothricin 92.1 per cent.

When infused with a 1 to 1,500 concentration of acriflavine in 20 per cent sucrose solution, 16 lactating quarters infected with *Str. agalactiae* and 1 infected with *Str. uberis* responded to treatment. To free them of the infection, 12 quarters required 1 infusion, 1 quarter 2 infusions, and 4 quarters 3 infusions. The milk of 1 quarter failed to return to pretreatment condition; the quarter became enlarged and showed evidence of abscess formation. *Str. agalactiae* infection was eliminated from 3 of 4 quarters treated dry. Two of these quarters produced abnormal milk for a considerable length of time after freshening.

One of 2 quarters infused with a 1 to 4,000 solution of aqueous acriflavine during the dry period was freed of *Str. agalactiae* infection. This quarter freshened with a severe case of staphylococcal mastitis and dried up completely in a few weeks. The other quarter developed acute streptococcal mastitis at freshening and produced practically no milk in the following lactation.

Eight lactating quarters treated with a 1 to 4,000 concentration of acriflavine in 20 per cent sucrose were freed of *Str. agalactiae* infection. Four quarters responded to 1 infusion, 2 quarters to 2 infusions, and 2 quarters to 3 infusions. Three treatments failed to eliminate the infection from 3 quarters. Two of the quarters that responded to the treatment produced abnormal milk until the end of the lactating period. One quarter treated while dry was free of *Str. agalactiae* infection after calving.

Entozon, in a 1 to 1,250 aqueous solution, eliminated *Str. agalactiae* from 16 lactating quarters, from 1 quarter infected with *Str. uberis*, and from 1 quarter carrying an infection of unidentified streptococci. Four quarters responded to 1 treatment, 11 to 2 treatments, and 3 to 3 treatments. Twelve quarters infected with *Str. agalactiae* failed to respond to 3 infusions. Two quarters produced abnormal milk after elimination of the infection. Seven quarters infected with *Str. agalactiae* and 1 with *Str. uberis* responded to treatment during the dry period. Three quarters treated dry freshened with *Str. agalactiae* infection still present. Marked atrophy of 5 quarters resulted after the use of this agent.

Colloidal silver oxide eliminated *Str. agalactiae* from 41 lactating quarters, *Str. uberis* from 3 quarters, and an unidentified Streptococcus from 1 quarter. Eighteen quarters were cured with 1 injection, 9 with 2 injections, 17 with 3 injections, and 1 with 4 injections. One quarter failed to respond to 1 injection, 16 to 3 injections,

and 1 to 6 injections. Thirteen of 22 quarters injected at 24-hour intervals for three days responded to treatment. After elimination of the infection, 3 quarters produced abnormal milk for the remainder of the lactation. In treating 66 dry quarters infected with *Str. agalactiae*, 8 with *Str. uberis*, and 9 with unidentified streptococci, 58 responded to treatment. Twenty

TABLE 3—Relation of Induration to Efficiency of Chemotherapy

Classification*	Lactating Quarters			Dry Quarters		
	No. Examined	Freed of Infection (No.)	(%)	No. Examined	Freed of Infection (No.)	(%)
No Fibrosis	1	1	100.0	5	5	100.0
Slight Fibrosis	39	33	84.6	47	38	80.9
Distinct Fibrosis	79	54	68.3	61	53	86.9
Marked Fibrosis	29	14	48.3	17	13	76.5

*Classification according to physical examination.

quarters with *Str. agalactiae*, 2 infected with *Str. uberis*, and 3 with unidentified streptococci failed to respond. Increased amounts of 13, 14, and 15 cc. were injected into 24 quarters and 9 freshened free of infection. Alkaline milk was produced by 4 of the quarters treated dry after elimination of the offending organism.

Str. agalactiae infection was eliminated from 17 lactating quarters and *Str. uberis* from 1 quarter with tyrothricin. Sixteen quarters responded to 1 treatment and 2 quarters responded to 2 treatments. Seventeen quarters infected with *Str. agalactiae* failed to respond to this agent. The infection was not eliminated in 3 quarters treated once, 3 quarters treated twice, and 11 quarters treated three times. Of these, 9 quarters were treated while showing symptoms of acute mastitis and in 8, the infection was not destroyed. Three quarters produced abnormal milk following elimination of streptococcal infection. In treating 41 dry quarters infected with *Str. agalactiae*, 6 with *Str. uberis*, and 4 with unidentified streptococci, the infection was eliminated from all quarters except 4 infected with *Str. agalactiae*. Thirty-four quarters responded to 40-mg. amounts of the agent and 13 responded to 80-mg. amounts. Four quarters failed to respond to the injection of one 40-mg. treatment. Four quarters developed acute cases of staphylococcal mastitis at the time of freshening and produced abnormal milk for several weeks. These quarters received 80-mg. amounts of the drug.

Following treatment, *Sta. aureus* appeared in 36 (23.1%) of the lactating quarters and in 26 (17.1%) of the quarters treated during the dry period. The infection appeared in 28.6 per cent of the quarters after treatment with acriflavine, 24.4 per cent of the quarters with Entozon, 15.8 per cent of the quarters with colloidal silver oxide, and in 22.1 per cent of the quarters treated with tyrothricin. On an individual herd basis, 17.4 per cent of the treated quarters became infected with *Sta. aureus* in herd A, 35.6 per cent in herd B, 19.3 per cent in herd C, 36.8 per cent in herd D, 16.7 per cent in herd E, and 2.2 per cent in herd F.

Discussion

The results of treating cows for streptococcal mastitis with these agents are somewhat similar to those reported by other authors. Streptococci were eliminated from 73 per cent of the quarters treated either during lactation or during the dry period. The highest percentage of cures (77.6%) occurred during the dry period, exceeding by nearly 10 per cent those treated while milking. However, there was some variation between the results of the use of the 4 agents in both milking and dry quarters.

Some of these variations can be accounted for possibly by differences in the conditions under which the agents were used. Among those may be the extent of damage to the quarter as represented by induration, activity of the disease in the quarter, the type of infection, the stage of lactation, and others not well understood. The influence of induration of the quarter upon the efficacy of the treatment is illustrated in table 3. It will be observed, particularly in the treatment of lactating quarters, that as fibrosis increases, the number of quar-

ters freed of infection decreases. Because of the difficulty in detection of any but marked induration in dry quarters, close agreement would not be expected. Undoubtedly, infection disseminated widely through the quarter, as indicated by extensive induration or fibrosis, cannot be reached as readily by medication as infection localized in the cistern.

It is logical to assume that quarters in which no macroscopic evidence of mastitis is present would respond more favorably to treatment than those producing abnormal milk or showing acute symptoms. The latter point is illustrated in the results with tyrothricin. Of 9 quarters treated during an attack of acute mastitis, only 1 was cured. Had these cases not been included in the results for this product, the percentage of quarters cured would be 65.3 per cent instead of 51.4 per cent. Although the number of quarters in each category that have been treated is too small to be conclusive, a definite trend toward lower therapeutic efficacy has been observed in quarters producing abnormal milk. This condition has been found with all products used, and the decrease has been about 15 per cent.

Somewhat closely allied with the influence of the activity of the disease in the quarter is the matter of the type of *Str. agalactiae* present in the herd. Since the early work of Lancefield[24] and Stableforth,[25] and the later work of Stewart,[26] several different types have been recognized in serological group B. Among these types are the so-called epidemic strains and others that cause comparatively little disturbance in the quarter. Although nothing has been reported as to the relationship between type and treatment, there seems to be no reason to believe that certain types of *Str. agalactiae* might not be more resistant to therapy than others. This fact could account for some of the variation in the results that Schalm[9] has observed when the same agent is used in different herds. Somewhat similar results have occurred in this work. With colloidal silver oxide used in 6 herds, the percentage of quarters cured ranged from 50 to 100 per cent, a variation of 50 to 92 per cent with tyrothricin in 4 herds, from 50 to 94.4 per cent with acriflavine in 4 herds, and from 50 to 80 per cent with Entozon in 4 herds.

Not so evident is the influence of the stage of lactation, other than the previously mentioned difference between the results of treating dry and lactating quarters. However, it appears that somewhat better results might be expected from the treatment of quarters during the later stages of lactation. This at least has been the general observation in this work, and may be due in part to the lower degree of dilution with milk while the agent remains in the gland.

On the other hand, the stage of lactation appeared to have little bearing on the irritant effect of the agents on the quarters during and after treatment. Varying degrees of swelling and secretion of abnormal milk resulted from the use of all the agents, irrespective of whether treatment was administered early or late in the lactation period. Nor was any appreciable difference noted in the time required for the clinical signs to leave the quarter. From the extent of the reactions induced by the several products, it appeared that tyrothricin caused comparatively little swelling in the quarter and changes in the milk. More swelling and pronounced changes in the milk occurred with colloidal silver oxide. Entozon was somewhat more irritating than colloidal silver oxide, and acriflavine, either in aqueous or sugar solutions, was the most irritating of the 4 used. In the case of acriflavine and Entozon at least, a part of their irritant properties might be derived from the large quantity of fluid infused. However, individual susceptibility may also be of some significance in the variable reaction of different quarters to these agents.

Unfortunately, the duration of this work has not been sufficiently long to determine definitely whether the irritant effect of treatment is reflected by permanent damage to the quarter in the form of increased induration and lowered milk production. From the figures now available, it does not appear that there is an unusual increase in induration of the quarter. Milk production,

on the other hand, was somewhat adversely affected, at least for the remainder of the lactation. There was an average decrease of about 16 per cent, with a range of from 12.1 to 36.7 per cent. The amount of the decrease correlated directly with the extent of irritation produced by each agent. It should be pointed out that these observations are based upon a small number of quarters and can hardly be considered conclusive.

An additional point that should be mentioned is the rather high incidence of infection with *Sta. aureus* in the treated quarters. This result has been noted by Schalm,[9] also. In 4 herds, the incidence of post-treatment staphylococcal infection was related rather closely to the extent of infection present in the herd at the time of the first bacteriological examination. In 2 other herds, however, the smaller number of infections occurred in the herd with the higher pretreatment incidence. Although no figures are available concerning the rate of spread of this infection in the individual herds, the number of quarters in which *Sta. aureus* developed after treatment appears unusually high. This occurrence can be attributed to no apparent reason other than an increased susceptibility of the quarter due to treatment. In view of the serious potentialities inherent in staphylococcal infection of the udder, this result raises certain points that must be considered in the final evaluation of the use of chemotherapeutic agents in the treatment of bovine mastitis.

SUMMARY

1) Acriflavine, Entozon, colloidal silver oxide, and tyrothricin have been tested in the treatment of streptococcal bovine mastitis. These agents were used in 6 dairy herds having a total of 485 milking cows.

2) The first bacteriological examination indicated that 166 (34.2%) of these animals were infected in 394 quarters with *Streptococcus agalactiae* and 32 (6.6%) with *Streptococcus uberis* in 48 quarters. *Streptococcus dysgalactiae* was present in 1 quarter, *Streptococcus fecalis* in 5 quarters, and unidentified streptococci in 39 quarters. *Staphylococcus aureus* infection was found in 103 (21.2%) of the animals on the initial examination.

3) Altogether, 106 (67.9%) of the lactating quarters responded to treatment, and 118 (77.6%) of the quarters treated during the dry period were found free of streptococcal infection after freshening. In the lactating quarters, acriflavine was found to have an efficiency of 89.3 per cent in destroying streptococcal infections, Entozon 60 per cent, colloidal silver oxide 71.4 per cent, and tyrothricin 51.4 per cent. In dry quarters, the efficiency of acriflavine was 71.4 per cent, Entozon 72.7 per cent, colloidal silver oxide 69.9 per cent, and tyrothricin 92.1 per cent.

4) There are several factors which obviously have a bearing on the results, such as the amount of fibrosis in the quarter, the activity of the disease, the type of infection, the stage of lactation, and others not well understood.

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Histopathology of Anasarcous Lesions Observed in Natural Cases of Vitamin A Deficiency in Cattle

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ANASARCA, commonly defined as a generalized or universal edema of the subcutaneous and intermuscular connective tissues, is now recognized as one of the more common clinical evidences of vitamin A deficiency in cattle.

We find, however, few references to this condition by the earlier investigators of vitamin A deficiency in cattle. Bechdel and his coworkers[1] were among the first to mention subcutaneous edema, which was observed in experimental cases of vitamin A deficiency in heifers. Some years later, Guilbert and Hart[2] reported subcutaneous edema, also in an experimental case. More recently, Schmidt[3] has reported edematous lesions in both experimental and field cases of vitamin A deficiency in cattle, and Hastings,[4] in his published observations on field cases of vitamin A deficiency in cattle, states that subcutaneous edema occurs quite regularly in such cases.

We have found little comment and few suggestions in published articles regarding the probable cause of anasarca in vitamin A deficiency in cattle, particularly as to whether the edematous condition is of cardiac or renal origin. Guilbert and Hart[2] suggest that the edema may be concerned with renal involvement. Hastings[4] simply states that the regularly occurring edema in vitamin A deficiency in cattle would seem to indicate that this vitamin is vitally concerned in the cutaneous circulation. He does not clarify his meaning on this point.

The writers do not feel that the limited studies made in these cases would justify an opinion regarding the probable causation of the edema or associated tissue changes, which doubtless will be revealed through more extensive studies of such cases in the future.

In a previous article,[5] reference was made to the increased incidence of vitamin A deficiency in cattle, particularly in feedlot animals, as evidenced by the increase in the number of cases showing anasarca reported from slaughtering establishments under federal inspection. It was material from such cases, forwarded by inspectors for laboratory diagnosis, which afforded the opportunity for making the studies here reported.

We have found only a limited number of references in the literature to histopathological studies made of the tissues in vitamin A deficiency in cattle. Langham and his associates[6] made histological studies of the kidneys of calves in experimental cases of vitamin A deficiency, and Thorp and coworkers[7] reported their observations on the pathology of various tissues, also in calves on a low vitamin A diet. Possibly others have made similar studies, but among the writers who have mentioned subcutaneous edema, or anasarca, as a symptom of vitamin A deficiency in cattle, we have found no description of the histopathology of such lesions.

Since the chief gross lesion observed on postmortem inspection in advanced cases of vitamin A deficiency in cattle is the extreme edema (anasarca) of the subcutaneous and muscle tissues, these were the tissues always submitted by inspectors for laboratory examination. They were not accompanied by other tissues or organs with any degree of regularity. For this reason, therefore, and also because of the fact, as previously indicated, that we have found no references to previous reports on the histopathology of such cases, our findings as reported here will be limited largely to the tissues mentioned.

From the Bureau of Animal Industry, Agricultural Research Administration, U. S. Department of Agriculture.

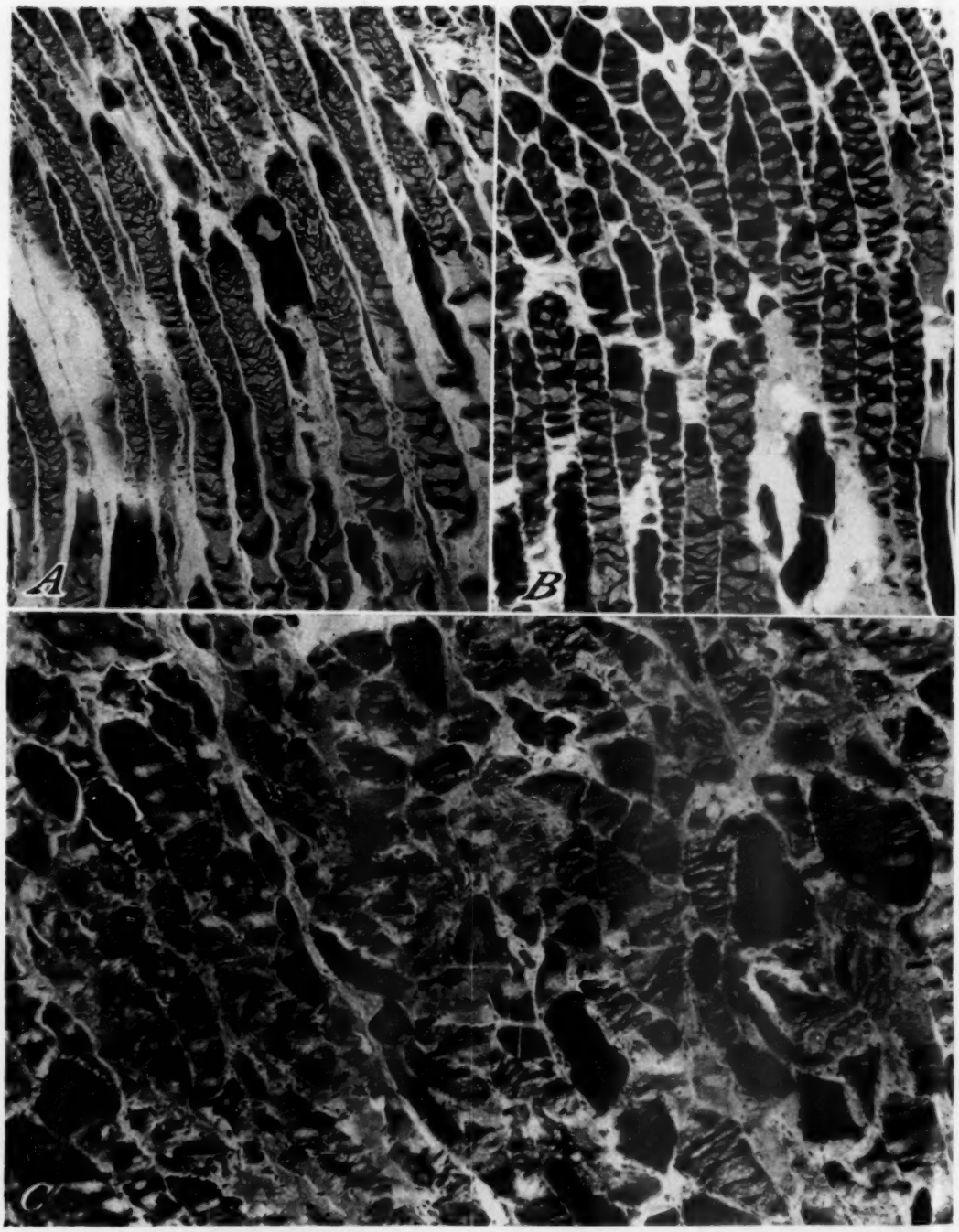

Fig. 2—Sections of muscle tissue from three animals, A, B, and C, illustrating the similarity of the degenerative changes seen in the edematous muscle of the different cases. x100.

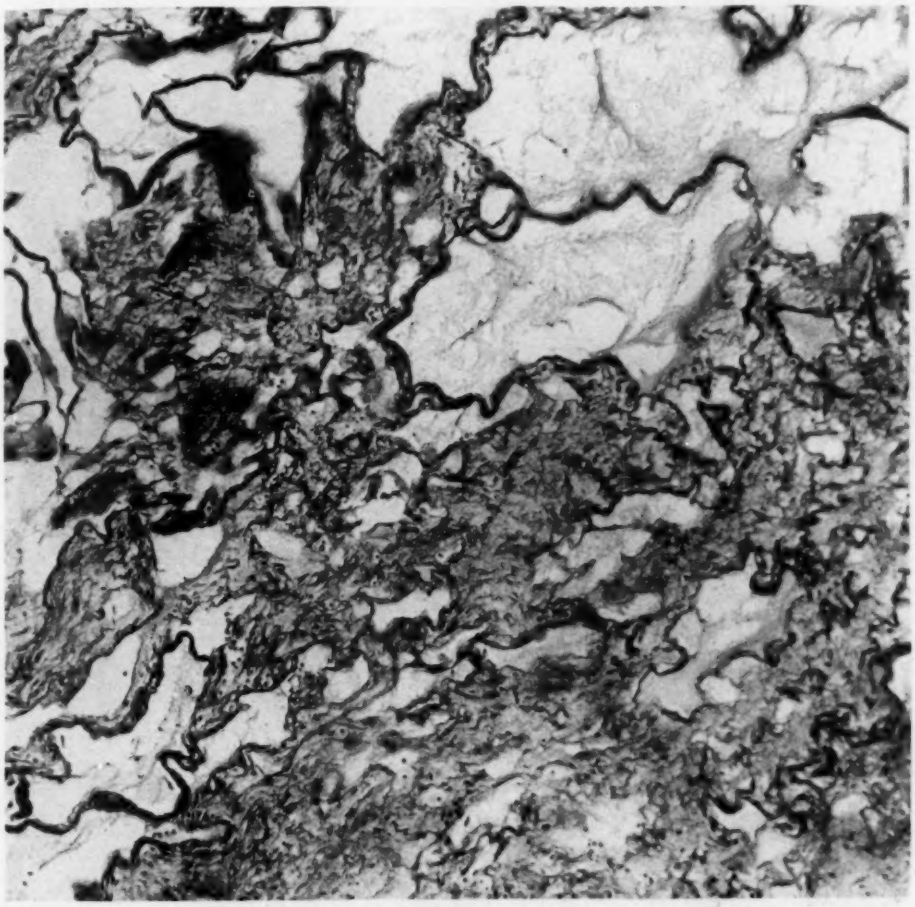

Fig. 1—Section showing the characteristic extensive edema of the subcutaneous tissue. x100.

Gross Pathology.—Before giving the histopathology in these cases, possibly it would be of some interest to describe briefly the gross pathology observed *post mortem* in the advanced cases of vitamin A deficiency, in which there is well-marked clinical evidence of anasarca. On removal of the skin from the carcass, there is first observed a serogelatinous exudate infiltrating the subcutaneous structures and underlying musculature, and much straw-colored fluid is seen oozing from the tissues. In some cases, the serous fluid will continue to drip from the affected carcass for a number of hours. In one particular case which came under our observation, the serogelatinous exudate was seen extensively infiltrating the deeper structures beneath the scapula, involving the nerve trunks of this region which were noticeably swollen and edematous. An unusual prominence of the peripheral arteries and arterioles was also noticed in this case. The body lymph glands are usually somewhat swollen, edematous, more or less congested, and may be hemorrhagic.

Histopathology.—Considerable similarity was found in the histopathology of the subcutaneous and muscle tissues from some 12 different cases examined. The outstanding change in all cases was the extreme and extensive edema of the involved tissues. The edema of the tissues was accompanied by heavy deposits of fibrinous and pink-stained granular material, probably protein substance (fig. 1). Varying numbers of cellular elements were infiltrating the edematous tissues. These consisted of lympho-

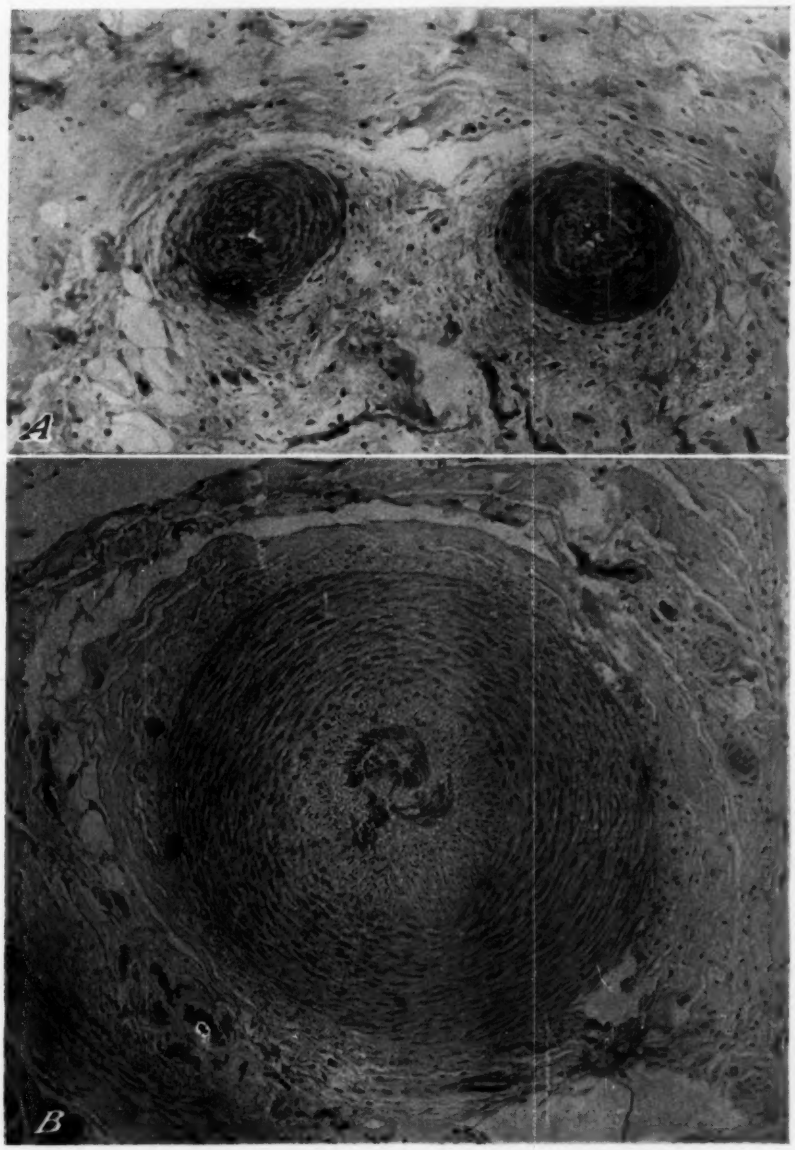

Fig. 3—Intimal thickening and almost complete occlusion in peripheral arterioles of the subcutaneous tissue A and also in artery of the edematous muscle B. x100.

cytes, plasma cells, neutrophile leucocytes, eosinophiles, and occasional histiocytes. Free red cells were also seen rather frequently in the edematous areas and small hemorrhages were noted occasionally. In some cases, there was a tendency to organization of the fibrinous material, more particularly in the intermuscular connective tissue, and in these cases which appeared to be of long standing rather definite fibroblastic changes were noted. In many areas in the edematous tissues, the peripheral arterioles and capillaries were more or less tortuous in appearance.

There was a particularly striking similarity in the degenerative changes observed in the muscle tissue in the different cases. It seemed evident that, as a result of the long continued presence of excessive fluids in the tissue, the muscle had undergone very marked hydropic and atrophic changes. The changes varied somewhat in degree in the different portions of the muscle affected. In the earlier stages of the degen-

Fig. 4—Showing rather numerous elastic fibers mixed with the collagen fibers in the thickened intima of vessel A, while in the larger vessel B few elastic fibers can be seen. Compare artery B with normal vessel C. Weigert's elastic tissue stain. x100.

erative process, there were vacuolation of the fibers, loss of striations, fibrillar separation, and segmentation. In the more advanced degenerative changes, there were marked clumping and hyalinization, with practically complete disintegration of many fibers, only remnants of which could be seen in many areas (fig. 2: A, B, and C).

The vascular lesions in these cases, so far as could be determined, seemed to involve the peripheral vessels largely, or the smaller arteries, arterioles, and capillaries. The changes were thickening of the walls of the vessels, some of which showed distorted and eccentric lumens, and many practically complete occlusions (fig. 3: A and B).

The thickening of the walls of the arterioles involved chiefly the intima, with evidence of hypertrophic changes in the media of some vessels. Apparently, the adventitia was not involved. As determined by special staining methods, it was evident that the thickenings of the intima were due largely to hyperplasia of its connective tissue elements or increase in the concentrically arranged collagen fibers. In those vessels showing the more marked thickening, varying numbers of elastic fibers were seen mixed with the collagen fibers (fig. 4: A and B). Hyalin changes were present in some vessels. Judging from the various cases examined, it seemed evident that a large percentage of the peripheral vessels were involved in the hyperplastic changes.

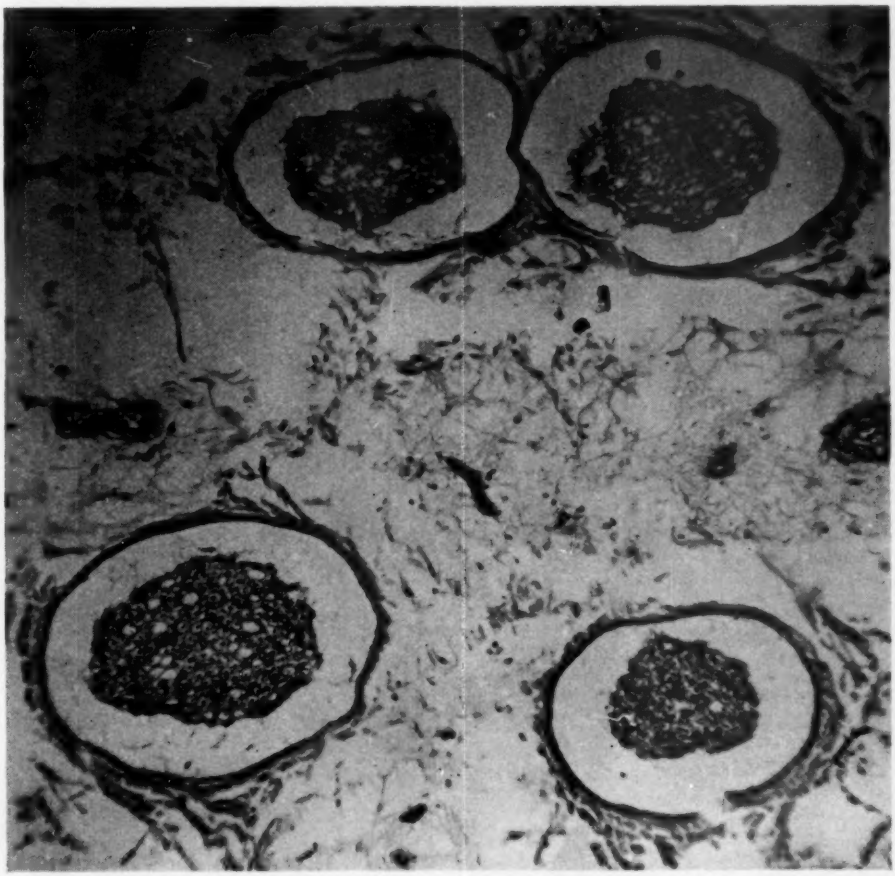

Fig. 5—Edema and atrophic changes as seen in the peripheral nerves in the anasarcous lesions. x100.

Sections of tissues which included the peripheral nerves showed well-marked evidence of edema of these structures, with

atrophic changes and greatly increased space within the surrounding sheath (fig. 5). The body lymph glands were examined in a few cases. The changes in these consisted of edema, much distension of the lymph vessels, congestion, and occasional hemorrhages. In a few instances, specimens of heart muscle were submitted for examination. In most of these, the myocardium appeared more or less hydropic and in 1 case there were congestion and a few hemorrhages. There was also evidence of thickening of the walls of arterioles in several heart sections. Other organic tissues, such as liver and kidney, were received only in a limited number of cases. Sections from these organs either revealed no specific changes, or were found unsatisfactory due to slight postmortem changes in the tissues.

SUMMARY

A description is given of the histopathology of well-marked anasarcous lesions in 12 natural cases of vitamin A deficiency in cattle.

In these cases, certain tissue changes were observed which were common to all. These were the marked and extensive edema, muscle degeneration, and vascular alterations.

The tissues examined in the different cases were limited largely to the edematous subcutaneous and muscle tissues. Therefore, with a few exceptions, no attempt has been made to include organic and other tissues in our histological findings in these cases.

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Observations on the Normal Estrous Cycle and Breeding Season in Goats and Possibilities of Modification of the Breeding Season with Gonadotropic Hormones

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PRODUCERS of goat's milk are faced with a difficult problem of maintaining a fairly uniform level of production throughout the year. The breeding season is limited and the average doe cannot be milked profitably for more than five or six months.

Possible means of spreading the period over which does come in milk include the following:

1) Delay breeding of a portion of the does so that some are bred at different times during the breeding season.

2) Select for does that tend to come in estrus outside the usual breeding season, thereby increasing the spread in possible breeding dates.

3) Stimulate extra-seasonal breeding by artificial means, such as by treatment with gonad-stimulating hormones and by variations in the amount of light.

4) Stimulation of lactation, without gestation, by the use of lactogenic hormones.

Preliminary work by Bissonette[1] has indicated the possibility of stimulating extra-seasonal breeding in goats by reduction of light during the early part of the summer, and preliminary work by Lewis and Turner[2] and Folley and Young[3] points to the possibility of inducing lactation in virgin goats by injections of stilbestrol, a synthetic chemical product. Further work is needed before these procedures can be recommended for application by goat breeders.

Kupfer[4] studied the ovaries of Swiss goats and found that ovulation definitely occurred during the months of October, November, and December. He states that the breeding season may extend to the adjoining months under certain circumstances, such as under the influence of domestication. He also states that ovulations occur at regular intervals of three weeks, and describes the cyclic changes in the ovaries. The breeding season or period of ovulation is followed by a period during which ovulation is completely suppressed. Similar observations were made on Boer and Angora goats in South Africa and the findings were similar, except that the breeding season was approximately from April to August, due to the reversal of seasons as compared with the northern hemisphere.

Sandburg[5] has reported some success in stimulating extra-seasonal breeding by injections of Gonadin, a gonadotropic hormone prepared from the blood serum of pregnant mares. She also states that some Nubian breeders have been very successful with out-of-season breedings, using no artificial means of stimulation, but no data are given.

The data reported in this paper were collected in an attempt to obtain information on the duration of the breeding season, the length of the estrous cycle, and on the possibilities of obtaining pregnancies in does injected with gonadotropic hormones outside the usual breeding season.

MATERIALS AND METHODS

Saanen and Toggenburg does and kids in the Bureau of Animal Industry's herd at the Beltsville Research Center, Beltsville, Md., were used in the study.

Observations on the occurrence of estrus were made daily, using bucks as teasers. They were begun on Oct. 20, 1941, and the data used in this paper were obtained from that date to Dec. 13, 1942. In addition to the regular milking does and developing kids, the herd contained a group of dry does that had been

From the Animal Husbandry Division, Bureau of Animal Industry, Agricultural Research Administration, U. S. Department of Agriculture.

The authors acknowledge helpful advice and assistance of Dr. R. M. Fraps in the work on stimulation of extra-seasonal breeding with hormones.

culled from the breeding herd for various reasons but which were being used in other experimental work. The total numbers observed for estrus included 32 milking does, 20 dry does, 16 kids born in 1941, and 19 kids born in 1942.

In the work with harmones, 15 dry and 5 milking does were used. The hormone products used included the following products: "Gonadin Serum" of equine origin, produced by Cutter Laboratories,* and containing 50 rat units per cc., and luteinizing hormone produced by Difco Laboratories,† an extract from the anterior pituitaries of sheep. One mg. of the luteinizing hormone was equivalent to 1 rat unit (Fevold seminal vesicle test).

*Berkeley, California.

†Detroit, Michigan. The authors are indebted to the Difco Laboratories for providing this material.

DATA AND DISCUSION

The Breeding Season.—Females of breeding age or approaching breeding age were observed daily for estrus from Oct. 20, 1941, until Dec. 13, 1942, with the exception that observations on does in the milking herd did not begin until these does freshened in the spring of 1942. The results are summarized in figure 1. The data were arranged by 21-day periods in order to show the relative number of does showing estrus at different seasons. The total column for each 21-day period indicates the number of individuals actually under observation during that period. The solid portion of the column indicates the number that actually showed estrus during the pe-

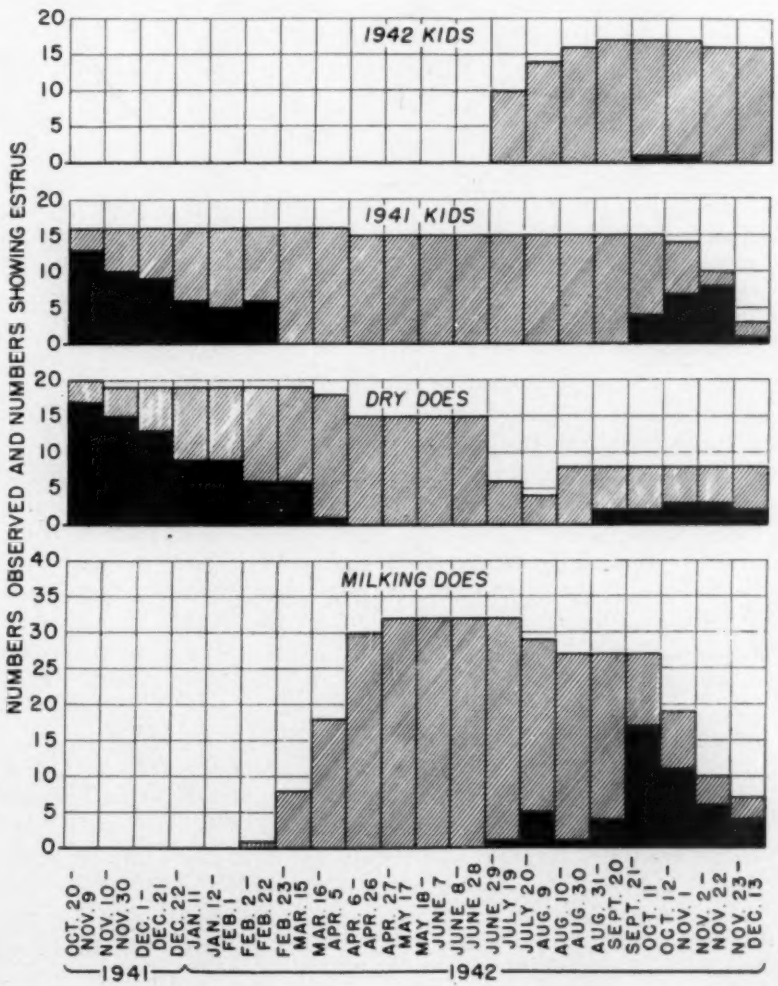
1942 KIDS
1941 KIDS
DRY DOES
MILKING DOES
NUMBERS OBSERVED AND NUMBERS SHOWING ESTRUS
OCT. 20 - NOV. 9
NOV. 10 - NOV. 30
DEC. 1 - DEC. 21
DEC. 22 - JAN. 11
JAN. 12 - FEB. 1
FEB. 2 - FEB. 22
FEB. 23 - MAR 15
MAR 16 - APR. 5
APR. 6 - APR. 26
APR. 27 - MAY 17
MAY 18 - JUNE 7
JUNE 8 - JUNE 28
JUNE 29 - JULY 19
JULY 20 - AUG. 9
AUG. 10 - AUG. 30
AUG. 31 - SEPT. 20
SEPT. 21 - OCT. 11
OCT. 12 - NOV. 1
NOV. 2 - NOV 22
NOV. 23 - DEC. 13
1941
1942

Fig. 1—The breeding season in goats. Shaded columns indicate number of females under observation in each 21-day period. Black columns show the number of females that actually came in estrus in each period.

riod. If a doe came in estrus twice during the period she was included only once in the tabulation. The observations on each group of does is discussed below.

The milking does began kidding in February, and the number under observation for estrus increased from that point as the season advanced until kidding was over. No estrus was observed until July 15, this being the one individual recorded in figure 1 as showing estrus between June 29 and July 19. In the making of the tabulations in table 1, each doe that was bred was removed from the tabulation after the period in which she conceived, hence the number under observation gradually decreases in the milking group and in the 1941 kids during the 1942 breeding season an occasional animal was also removed because of death. The majority of the does did not begin coming in estrus until the latter part of September.

TABLE 1—Summary of Data on Estrous Cycles in Three Groups of Goats

Group	Cycles Observed (No.)	Average Length (Days)	Standard Deviation (Days)
Dry does....	71	22.8	13.7
Milking does	29	23.0	19.7
1941 kids ...	61	16.6	14.6

Most of the dry does exhibited estrus during the first period when observations were begun in October, 1941, and the number gradually declined until the period March 16 to April 5, when only one doe came in estrus (on March 18). These data indicate that if breeding were postponed beyond about the middle of November, the chances of finding all the does in estrus would decrease progressively, and that if breeding were delayed beyond about the middle of December, some of the does held over for late breeding might go unbred. Data such as these are rare since dry herds of goats of the milking breeds are seldom maintained for so long a period without being bred, but further data should be obtained on this point, under various conditions, to determine more definitely the limits of equalizing the milk supply through delayed breeding in a portion of the herd. Only a few of the dry does were available for observations on the normal occurrence of estrus in the fall of 1942 since most of them were used in studies with hormones, reported later.

The data on mature does indicate that a few does could be bred during late July and August, but that most breeding in the herd studied would need to be done between mid-September and mid-December, a period of three months, thus limiting the extent to which the time of freshening can be spread by breeding some does early and some late in the breeding season.

The kids born in 1941 were coming into estrus at about the same rate as were the dry does when observation began, but the breeding season did not extend so far into the winter. The first occurrence of estrus in the following fall was considerably later than the first estrus among the milking does. These observations indicate that the anestrous period after the kid's first breeding season may be considerably longer than in older does.

The kids born in 1942 differed markedly from those born in 1941, in that only two had exhibited estrus up to December 13. They were born at about the same time and had been given comparable care, hence no reasonable explanation can be given for the difference in the two groups. The 1942 kids were placed under observation at 4 months of age.

Another approach was made to the problem of extent of the breeding season by analyzing date of birth of kids from 100 does in each of three breeds, Nubian, Saanen, and Toggenburg. These data were taken from volume 59 of The American Goat Record Association Registrations issued in 1940. The first 100 purebred registrations in each breed were selected, with the exception that animals from the Bureau of Animal Industry's herd at Beltsville, Md., were excluded to avoid duplication of animals used in other phases of this study. The date of successful breeding was assumed to be five months prior to date of kidding. The proportion of the does in each breed that were settled in each month is indicated in figure 2. No information is

available concerning attempts to breed these does out of season, but the data shown graphically in figure 2 do illustrate the extent to which the breeding season in these three breeds of goats is limited with present breeding practices. These data do not give a definite indication that does of one breed are being bred out of season more than any other of the three. However, 3 and something of the possibilities of improving this characteristic by selection.

The Estrous Cycle.—Estrous cycles were observed in the milking does, the dry does, and the 1941 kids. In the latter group, the cycles as kids and as yearlings were grouped together since the two distributions did not differ materially. The data are summarized in table 1. The figures

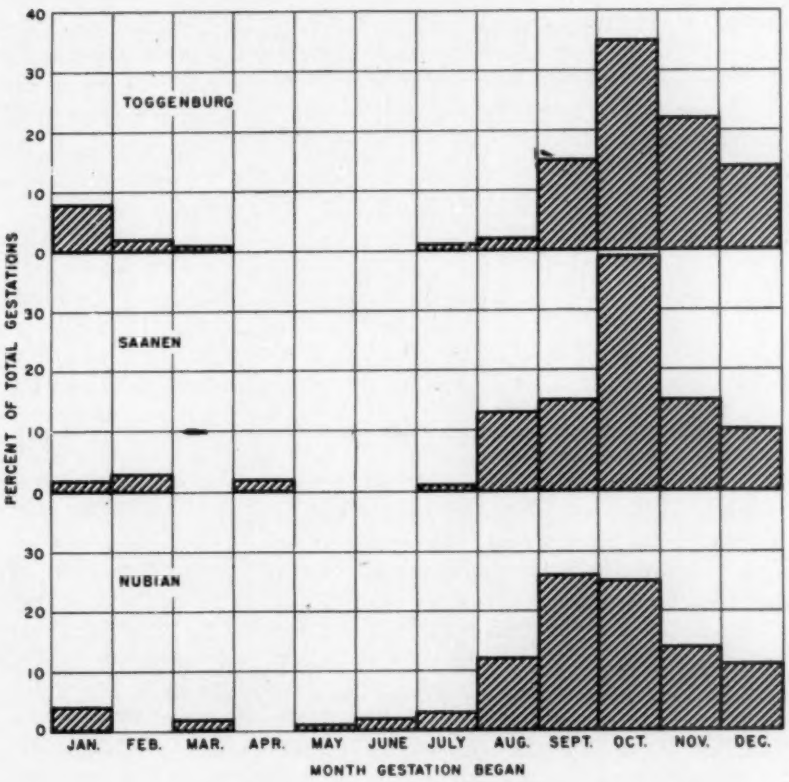

Fig. 2—The season at which goats are bred. Distributions show the time of conception for 100 does in each of the three breeds.

per cent of the Nubians were settled in May and June, as contrasted to none in the other breeds, and a greater proportion was settled in September in this breed than in the others, where the peak of successful breedings is in October. Whether one breed may have more potentialities for extra-seasonal breeding than another could only be determined by careful experimentation with adequate numbers of animals. Such information would be of value to goat breeders to indicate the possibilities of extra-seasonal breeding in existing stock given may be somewhat misleading unless they are interpreted in the light of distributions of the estrous cycles given in figure 3. The standard deviations given in table 1 indicate that the distributions are extremely variable and this is borne out by inspection of figure 3. Inspection of this figure indicates that the distributions tend to be trimodal. A considerable number of the estrous cycles fall in about twenty-one days, but only a relatively small number are actually of this length. A number of shorter cycles, twelve days or less, were

also observed, as well as some that were considerably longer. The longer cycles may well be multiples of cycles approximating twenty-one days in length. This type of cycle has been observed in other species, but the short cycles are more difficult to interpret. Obviously, there is wide variability in the length of the estrous cycle in the goats studied. This variability, particularly in the occurrence of what may be considered abnormally short cycles, is much more marked than has been found in sheep by McKenzie and Terrill.[6] Some of the very short cycles, such as those of only two to four days, might be interpreted as cases of split estrus, but studies of the ovaries in such cases would be necessary in order to make sure of this point.

Production of Estrus with Gonadotropic Hormones.—Ten dry does were used in the

TABLE 2—Summary of Results with Injections of Gonadotropic Hormones in Dry Does. (All injections were made subcutaneously unless otherwise noted)

Breed and No.*	Age (Yr.)	Weight (Lb.)	Dosage			Results			
			Date treated	P.M.S.† (Units)	L.H.‡	Date in estrus	Results of breeding		
							Date kidded	No. young	Date next estrus observed
T71	4	104	6/19	400	..	...	....	..	....
			7/9	200	..	...	....	..	....
			7/29	400	..	8/5, 6	....	..	9/17
T202	3	76	6/19	200	..	...	....	..	....
			7/9	200	..	...	....	..	....
			7/29	400	..	7/31	....	..	11/9
T220	3	86	6/19	200	..	...	....	..	....
			7/10	200	..	...	....	..	....
			7/29	400	..	8/2	....	..	9/20
			8/2	...	5‖	8/2			
T64	4	138	6/23	200	..	...	....	..	....
			7/13	400	..	7/16	12/14	1	....
			7/16	...	3‖	7/16			
T211	3	90	6/23	200	..	...	....	..	....
			7/13	400	..	7/16	....	..	None§
			7/16	...	3‖	7/16			
T247	2	114	6/23	200	..	...	....	..	....
			7/13	400	..	7/15, 16	....	..	11/3
T273	2	97	6/23	200	..	...	....	..	....
			7/13	400	..	7/15	12/14	3	....
T217	3	114	6/26	200	..	...	....	..	....
			7/16	400	..	7/24	12/24	1	....
T226	3	78	6/26	200	..	...	....	..	....
			7/16	400	..	7/17	....	..	11/9
T241	2	101	6/26	200	..	...	....	..	....
			7/16	400	..	7/18	12/17	2	....
			7/18	...	5‖	7/18			
S107	6	177	6/30	150	..				
			7/2	150	..				
			7/4	150	..	7/16	....	..	None§
			7/6	150	..				
			7/8	150	..				
			7/10	150	..				
T218	3	129	6/30	150	..				
			7/2	150	..				
			7/4	150	..	7/7	12/9	3	....
			7/6	150	..				
			7/7	...	5**				
T253	2	92	6/30	150	..				
			7/2	150	..				
			7/4	150	..	7/6, 7, 8	12/4	2	....
T209	3	75	7/18	400	..	7/21	....	..	10/15
T245	2	78	7/18	400	..				
			7/21	...	1‖	7/21	....	..	11/6

*T = Toggenburg, S = Saanen. †Pregnant mare serum, "Gonadin." ‡Luteinizing hormone. §No estrus during subsequent breeding season and up to Jan. 21, 1943. ‖Intravenous injection. **Intramuscular injection.

initial series of injections and were given 200 units of P.M.S.* None came in estrus as a result of the first injections. These are the first 10 does listed in table 2. According to plan, each doe was to receive a second dose twenty days later if she did not come in estrus prior to that time. The first 3 does were given 200 units each, but did not respond, so the second dose was raised to 400 units on the remaining 7 and all came in estrus in from one to eight days, and all but 1 within three days. The first 3 does were given a third dose of 400 units nineteen or twenty days after their second and all came in estrus within two to seven days.

Three dry does were injected with 150 units on alternate days, the plan being to continue these injections until a maximum of 6 had been given or until the does came in estrus. One doe came in after the third, 1 after the fourth, and 1 six days after the sixth injection.

Two additional dry does were injected with 400 units each and both came in estrus in three days.

These results indicate that 400 units of P.M.S. are adequate to bring dry does in estrus, whether or not any preliminary dose is given. The use of one dose of this size may be more economical than a series of smaller injections.

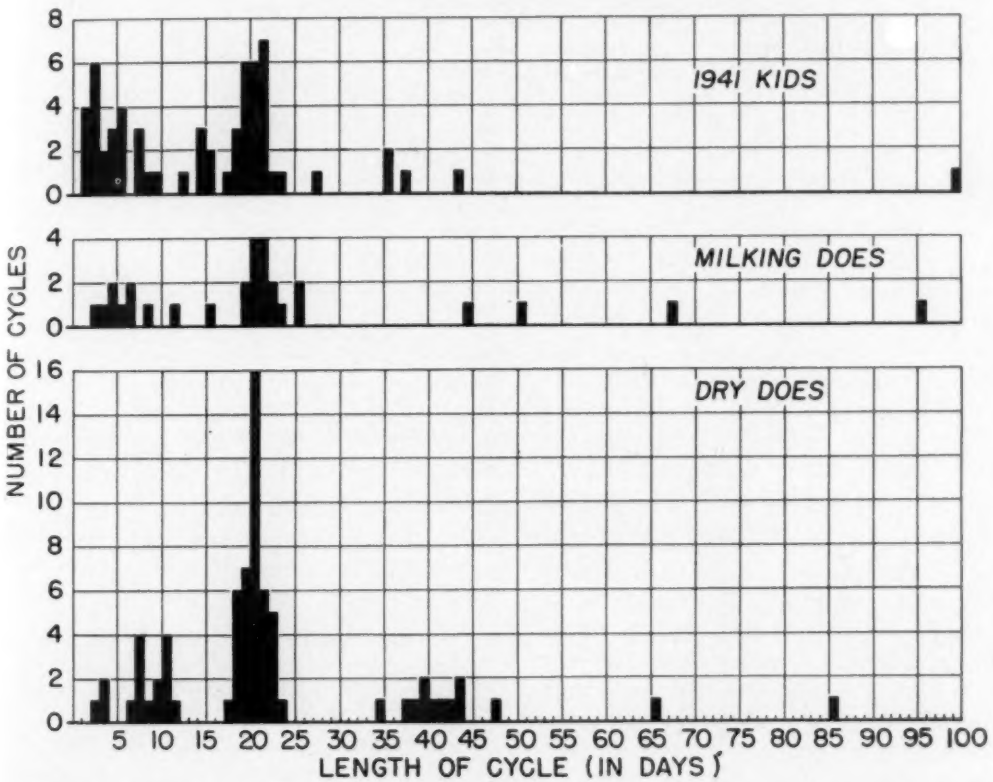

Fig. 3—Length of the estrus cycle in does. Distributions show the lengths of cycles observed in each of three groups of females.

Five lactating does were treated with doses of P.M.S. varying from 200 to 600 units. Doses of 400 units were adequate to produce estrus in 2 cases, and 2 does that failed to respond to 400 units came in estrus when given 600 units. One doe failed to respond to one 400- and two 600-unit doses.

All the does were bred while in estrus.

*Pregnant mare serum.

Of the 15 dry and 4 lactating does that came in estrus after injections of P.M.S., 6 of the dry does and 1 of the lactating does conceived and produced kids. These data are presented in tables 2 and 3. The numbers are too limited to serve as a basis for definite conclusions, but there is an indication that lactating does may not respond to treatment as readily as dry does. The does in the two groups that came in estrus but failed to conceive following the treatments with hormones came in estrus again at intervals of from 43 to 140 days later (with two exceptions) and these estrous periods were within the normal breeding season. The two exceptions were does that failed to come in estrus during the breeding season, and it is possible that the normal functioning of the ovaries may have been disrupted by the action of the hormones.

Seven of the 19 does that came in estrus after injections of P.M.S. were given injections of from 1 to 8 units of L.H.,* with the thought that it might help stimulate ovulation. Injections were made between 11:00 a.m. and 2:00 p.m. on the day in which the does were found in estrus when checked in the morning. Of these animals, 3 became pregnant, while of the 12 not so treated, 4 became pregnant. Numbers are too small to justify any conclusions, but there is at least a slight indication that the luteinizing hormone may be helpful in stimulating ovulation at the proper time, and thereby making pregnancies possible. Further work is needed with larger numbers of animals in order to develop techniques that can be recommended for general use in practice.

*Luteinizing hormone.

TABLE 3—Summary of Results with Injections of Gonadotropic Hormones into Lactating Does. (All injections made subcutaneously unless otherwise noted)

Breed and No.*	Age (Yr.)	Weight (Lb.)	Dosage			Results			
			Date treated	P.M.S.† (Units)	L.H.‡	Date in estrus	Results of breeding: Date kidded	Results of breeding: No. young	Date next estrus observed
T248	2	98	7/2	200	..	...	....	..	....
			7/22	400	..	7/24	....	..	12/11
T265	2	95	7/2	400	..	...	....	..	....
			7/22	600	..	7/24	....	..	9/24
T83	4	81	7/8	400	..	...	....	..	....
			7/28	600	..	...	....	..	....
			8/18	600	..	8/25	1/29	1	....
T243	2	99	7/8	200	..	...	....	..	....
			7/28	400	..	8/2	....	..	11/15
			8/2	...	5§	8/2			
T257	2	92	7/8	400	..	...	....	..	....
			7/28	600	..	...	....	..	....
			8/18	600	..	...	....	..	10/3

*T = Toggenburg, S = Saanen. †Pregnant mare serum, "Gonadin." ‡Luteinizing hormone. §Intravenous injection.

SUMMARY AND CONCLUSIONS

Observations on 87 female Toggenburg and Saanen goats in the herd at the Beltsville Research Center, Beltsville, Md., and records on 300 females listed by the American Goat Record Association indicate that most does will come in estrus regularly between about the middle of September and the middle of December. A few does come in estrus during July, August, and early September, and some does continue to come in estrus through February and March. An occasional doe exhibits estrus during the intervening months of April, May, and June. Thus, the season during which most does can be bred with reasonable certainty appears to be limited to about three months in the fall.

Observations on 161 estrous cycles showed that the average length of the cycle

was 22.8 and 23 days in dry and milking does, respectively, and 16.6 days in kids. There was considerable variation, however, and the greatest number of cycles fell between eighteen and twenty-four days. A considerable group also fell between two and twelve days. Some long cycles occurred which appeared to be multiples of the most frequently observed length.

Preliminary work with 20 does indicates that there is some possibility of stimulating the ovaries with gonadotropic hormones (P.M.S. and L.H.) outside the usual breeding season, thereby making it possible to provide a more uniform milk supply throughout the year. Further work is needed to develop a procedure that can be used with reasonable certainty in practice.

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Corrigenda

Two errors made by the authors in their manuscript entitled, "The Pathology of Bovine Pyelonephritis" by Frank Thorp, Jr., Robert F. Langham, C. F. Clark and E. R. Doll, published in the July, 1943, issue of the American Journal of Veterinary Research:

Page 247, table 4—the dagger sign in the fructose line for cases 6022 and 1820 should have been a plus sign in each instance.

Page 249, under summary—in the first sentence of the second paragraph, *uric acid* should have read *urea*.

Experiments in Resistance to Fowl Sarcoma, Strain 13

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Philadelphia, Pennsylvania

FOWL SARCOMA, strain 13, is readily transmissible from chicken to chicken by tumor transplants or by filtrate of tumor tissue. This spindle-cell type of tumor was described by Stubbs and Furth.[1] Injected locally, tumor transplants or filtrates produce tumors at the site of injection by stimulating connective tissue cells to proliferate. Injected intravenously, such materials stimulate the endothelium of the blood-forming organs, producing diffuse sarcomatosis of the liver, spleen, and bone marrow. This tumor has been carried through a long series of transfers. It yields a high percentage of "takes," but some chickens appear so resistant that there is a wide variation in the size of the tumors produced by local injection. Most injections were made intramuscularly into the breast or intravenously into the wing vein.

Besredka and Gross[2] found considerable difference in the development of fowl sarcoma according to whether the fowl was infected by subcutaneous or intradermal injection. They found that intradermal sarcomas developed slowly, frequently showing arrested growth. When small doses of inoculum were used, the resulting tumor was reabsorbed in a few cases. Fowl with reabsorbed intradermal tumors became refractory to further injections of sarcomatous material administered either intradermally or intramuscularly.

The possession of an easily transferable sarcoma prompted me to make some studies of resistance. Strain 13 usually kills, but quite frequently chickens survive for several months. Occasionally, a chicken is found which fails to develop a tumor even after two or three injections several months apart. There is also some variation in the incidence of takes in different lots of material.

The tumor material used for injection in these experiments was selected from chickens that had been injected intramuscularly and that had tumors growing in the breast muscles. Small portions of such tumors were removed aseptically and finely minced by cutting with small scissors. Small portions of Ringer's solution were added to prevent drying and to serve as a vehicle for the tumor suspension. The mincing allowed the tissue fluids to escape and also provided for some cells in the tumor suspension. Usually, about the same amount of Ringer's solution was added as there was tumor tissue, but no attempt was made, other than estimation, to obtain approximately a 50 per cent suspension. The fluid portion or suspension was drawn through a pledget of cotton into a small syringe through a hypodermic needle, with the cotton around the point to keep small particles of tissue from obstructing the needle. The final fluid for injection was composed of tumor tissue fluid and cells in Ringer's solution. The whole process of preparation was done in Petri dishes kept on a pan of ice to keep all materials as cool as possible. Such suspensions were injected as soon as possible after preparation. In some cases, the chickens were killed for removal of the tumor and in other instances, small portions of the tumor were removed surgically under ether anesthesia.

Experiment 1.—Five chickens (11 to 15, inclusive) were injected intramuscularly in each breast with 0.2 cc. of tumor suspension, strain 13, from chicken 298 on Feb. 17, 1939. Table 1 illustrates these injections.

The table shows that of these 5 chickens, 4 (11, 12, 13, and 14) developed tumors in forty-four, ten, fourteen, and thirty-nine days, respectively, and died in one year, forty-six days, twenty-four days,

This work was supported in part by special grant No. 521, Faculty Research Committee, Board of Graduate Education and Research, University of Pennsylvania.

From the School of Veterinary Medicine, University of Pennsylvania.

TABLE 1—Injection of Strain 13 from Chicken 298 Intramuscularly Into Each Breast, Feb. 17, 1939

Injected into Chicken No.				
11	12	13	14	15
Tumors 44 days Reinjected 5 months Wing, wattle Negative Reinjected 7 1/2 months Wing, wattle Negative Dead 1 year Breast +++	Tumors 10 days Killed Transfer 46 days ++	Tumor 14 days Dead 24 days +++	Tumor 39 days Reinjected 5 months Wing, wattle Negative Dead 6 months ++	Negative Dead 49 days Negative

++++ = Very large tumor; +++ = Large tumor; ++ = Medium-sized tumor; + = Small tumor.

and six months, respectively. Chicken 15 failed to develop any tumor and died after forty-nine days. Chicken 11, after developing a tumor in forty-four days, carried that tumor until its death. It was injected again five months after the first injection by the use of fresh tumor material injected intradermally into the wing and wattle. No takes were obtained from these injections, and it was again injected seven and a half months after the first injection. Negative again, it died one year after the first injection, showing only a breast tumor of large size.

Chicken 14 developed a tumor in thirty-nine days that grew rather slowly. It was injected again five months later by the intradermal method in the wing and wattle. The results were negative in these sites. The chicken died one month after the second injection with a medium-sized tumor in the breast.

Experiment 2.—Five chickens (43 to 47, inclusive) were injected intradermally in the left wattle with 0.1 cc. of tumor suspension, strain 13, from chicken 12 on Mar. 30, 1939. Table 2 illustrates these injections.

The table shows that 4 of these 5 chickens developed tumors at the site of injection in nineteen to twenty-six days. Three of these (45, 44, and 46) died in thirteen, twenty-one, and twenty-five days, respectively, after the tumors appeared. One (46) showed metastasis to the liver with a tumor the size of a walnut. No metastases were noted in 2. The fourth tumor chicken (47), that showed a small tumor twenty-six days after injection, carried this tumor until it was killed five months after its first injection. Three months after injection, while carrying a wattle tumor, it was again injected intramuscularly in each breast and intradermally in the skin of the left wing web with tumor material from chicken 105. These injections were negative and the bird was killed while still carrying the wattle tumor from the original injection, but with all other sites negative.

Chicken 43, the fifth of this series, was negative to the wattle injection. It was injected again three months later with tumor

TABLE 2—Injection of Strain 13 from Chicken 12 Intradermally Into the Left Wattle, March 30, 1939

Injected into Chicken No.				
43	44	45	46	47
Negative Reinjected 3 months later Breast, wing, wattle Tumor, breast, wattle Reinjected 2 1/2 months later Other wing, wattle Negative	Tumor 19 days Dead 21 days later ++++	Tumor 19 days Dead 13 days later +++	Tumor 19 days Dead 25 days later +++	Tumor 26 days Reinjected 3 months later Breast, wing Negative Killed 5 months Only wattle tumor +

++++ = Very large tumor; +++ = Large tumor; ++ = Medium-sized tumor; + = Small tumor.

material from chicken 105, intramuscularly in each breast and intradermally in the skin of the wing web and wattle. It developed a small tumor in the left breast aften ten days and a wattle tumor after forty days. The breast tumor remained small, while the wattle tumor disappeared after one month.

Experiment 4.—Five chickens (53 to 57, inclusive) were injected intramuscularly in

TABLE 3—Injection of Strain 13 from Chicken 12 Intradermally Into the Left Wing, March 30, 1939

Injected into Chicken No.				
48	49	50	51	52
Negative Dead 75 days Negative	Tumor 14 days Dead 43 days later Metastasis to liver +++	Tumor 12 days Dead 38 days later +++	Negative Dead 55 days Pecked Negative	Tumor 24 days Dead 17 days later +++

++++ = Very large tumor; +++ = Large tumor; ++ = Medium-sized tumor; + = Small tumor.

Two weeks later, or about six months after the first injection, this chicken again was injected intradermally in the skin of the opposite wing and wattle with tumor material from chicken 231. Each of these injections was negative.

Experiment 3.—Five chickens (48 to 52, inclusive) were injected intradermally in the skin of the left wing web with 0.1 cc. of tumor suspension, strain 13, from chicken 12 on Mar. 30, 1939. Table 3 illustrates these injections.

The table shows that 3 of these chickens (50, 49, and 52) developed tumors at the site of injection in twelve, fourteen, and twenty-four days, respectively, and died after thirty-eight, forty-three, and seventeen days, respectively, from the time the tumors first appeared. Two chickens (48 and 51) were entirely negative until 1 (48) died seventy-five days later and was negative, and 51 was badly pecked fifty-five days later and was killed without any tumors showing.

the breast muscles with 0.2 cc. of tumor suspension, strain 13, from chicken 12 on Mar. 30, 1939. Table 4 illustrates these injections.

The table shows that 4 of these chickens (54, 55, 56, and 57) had small tumors at the point of injection at the first examination, twelve days after injection. The other (53) developed tumors on the seventeenth day following injection. This chicken injured its back and was killed eleven days later when it showed a small tumor in each breast. Chicken 54 died seventy-two days after the tumor appeared when it showed a very large tumor in each breast. Chicken 57 was killed two days after a tumor was noticed, or fourteen days after injection. Chicken 55, that developed a small tumor twelve days after injection, continued to

TABLE 4—Injection of Strain 13 from Chicken 12 Intramuscularly Into Each Breast, March 30, 1939

Injected into Chicken No.				
53	54	55	56	57
Tumor 17 days Back injured Killed 11 days later Small tumor each breast +	Tumor 12 days Dead 72 days later ++++	Tumor 12 days Reinjected 3 months later Wing, wattle Small tumor Wing, wattle 40 days Dead 5 months +	Tumor 12 days Pecked Dead 44 days later ++	Tumor 12 days Killed for transfer 2 days later Metastasis to liver ++

++++ = Very large tumor; +++ = Large tumor; ++ = Medium-sized tumor; + = Small tumor.

carry small tumors in each breast until its death five months later. It was reinjected three months after the first injection, while it was carrying a small tumor in each breast. This reinjection was made intradermally into the skin of the wing and wattle with 0.1 cc. of tumor suspension from chicken 105. Small, pealike tumors developed in each location, but never became progressive.

Experiment 5.—Five chickens (103 to 107, inclusive) were injected intramuscularly in the breast with 0.2 cc. of tumor suspension, strain 13, from chicken 57, on Apr. 13, 1939. Table 5 illustrates these injections.

The table shows that of these 5 chickens, the first 3 (103, 104, and 105) each developed a tumor from the first injection. These tumors developed in nineteen to forty days. Chicken 105 was killed for transfer four days after the first tumor appeared; 103 died seventy-four days after the tumor appeared and showed a very large tumor in the right breast. Chicken 104 developed a small tumor in the left breast. Three months later, it was reinjected in the skin of the wing and wattle with material from chicken 105, but these injections remained negative. The small breast tumor disappeared and forty-seven days after the last injection, when the chicken died, no tumors were found in the breast, wing, or wattle.

Chicken 106 was negative to the first injection and three months later was reinjected intramuscularly in the breast and intradermally in the skin of the wing and wattle. All injections remained negative. The material injected, from 105, was proved virulent by injections into other chickens. Chicken 107 was negative to the first injection and was reinjected three months later, using material from chicken 105, by intramuscular injection into each breast and intradermally into the skin of the wing and wattle. Small tumors developed at the point of injection in each place after ten days. The tumor in the wattle remained

TABLE 5—Injection of Strain 13 from Chicken 57 Intramuscularly Into Each Breast, April 13, 1939

Injected into Chicken No.				
103	104	105	106	107
Tumor 19 days Dead 74 days later ++++	Tumor 40 days Reinjected 3 months later Wing, wattle Negative Dead 47 days later Negative	Tumor 40 days Killed for transfer 44 days later ++	Negative Reinjected 3 months later Breast, wing, wattle Negative Dead 3 weeks later Negative	Negative Reinjected 3 months later Breast, wing, wattle Small tumors all points 10 days Dead 52 days later Very large tumor ++++

++++ = Very large tumor; +++ = Large tumor; ++ = Medium-sized tumor; + = Small tumor.

TABLE 6—Injection of Strain 13 from Chicken 105 Intramuscularly Into the Breast, July 7, 1939

Injected into Chicken No.							
197	198	199	200	321	322	323	324
Tumor 10 days Killed 18 days later ++++	Tumor 10 days Killed 2 months later +++	Tumor 10 days Regression Reinjection 2 1/2 months later Negative	Tumor 10 days Dead 21 days later ++++	Tumor 10 days Reinjection 2 1/2 months Negative Dead 4 1/2 months later ++++	Tumor 10 days Dead 1 month later +++	Tumor 10 days Killed Transfer 1 month +++	Tumor 10 days Dead 24 days later ++++

++++ = Very large tumor; +++ = Large tumor; ++ = Medium-sized tumor; + = Small tumor.

small, about the size of a pea. The tumor in the wing grew to hickory nut size. The tumor in each breast grew rapidly until this chicken died fifty-two days later. Very large tumors involved each breast and there was metastasis to the liver, spleen, mesentery, and intestine.

Experiment 6.—Eight chickens (197, 198, 199, 200, 321, 322, 323, and 324) were injected intramuscularly in the breast with 0.2 cc. of tumor suspension, strain 13, from chicken 105, on July 7, 1939. This was the same source material that was used for reinjection of many other chickens carrying tumors. These injections were made to control this source material regarding virulence. The results of these injections are shown in table 6.

The table shows that all of these 8 chickens developed tumors in ten days. Within one month, 5 were either dead or in such poor condition that they were killed, and each showed very large or large tumors in the breast. Chicken 323 was killed for transfer one month after injection. Chicken 199 developed small tumors in each breast that regressed. It was reinjected two and a half months later intramuscularly and intradermally, but the injections were negative. Chicken 321 developed a tumor in each breast that grew rather slowly but continuously. Two and a half months later, it was reinjected by the intradermal method in the wing and wattle, but these injections were negative. It died four and a half months after this reinjection when it showed very large tumors in each breast but no tumors in the wing or wattle.

Experiment 7—Three chickens (231, 232, and 233) were injected intramuscularly in the breast with 0.2 cc. of tumor suspension, strain 13, from chicken 224, on Aug. 28, 1939. The results of these injections are shown in table 7.

TABLE 7—Injection of Strain 13 from Chicken 224 Intramuscularly Into the Breast, Aug. 28, 1939

Injected into Chicken No.		
231	232	233
Tumor 15 days Killed for transfer 15 days later ++++	Negative Reinjected Each breast, wing, wattle 1 month later Negative Dead 3 months later Negative	Tumor 15 days Reinjected Wing, wattle 1 month later Negative

++++ = Very large tumor.

The table shows that 2 of these 3 chickens developed tumors in fifteen days. One (231) was killed for transfer fifteen days later. In chicken 233, the tumor remained small. This chicken was reinjected one month later, intradermally in the wing and wattle. These injections remained negative. The third chicken (232) was negative. It was reinjected one month later, intramuscularly in the breast and intradermally in the wing and wattle. All of these injections remained negative.

DISCUSSION

The source materials for transfer in these 7 experiments were obtained from 4 different chickens. The materials used for reinjection purposes were obtained from 2 different chickens. These materials were tested and shown to be potent in producing tumors and were as nearly uniform in character as was possible.

These experiments yielded 12 chickens that were used for reinjection purposes to test the resistance of chickens to tumors. Ten of these were carrying a tumor when reinjected, while 2 were not. Originally, most of these were injected intramuscularly. One of these (43) was negative on the original injection, but when reinjected three months later, both intramuscularly and intradermally, developed tumors in the breast and wattle. This chicken was again injected intradermally two and a half months later and was negative. Another chicken (107) was negative to the original intramuscular injection. It was reinjected three months later, intramuscularly in the breast and intradermally in the wing and wattle, after which small tumors formed in each site of injection. The intramuscular tumors grew larger and it died fifty-two days later with very large tumors in each breast. Other chickens (11, 14, 104, 199, 321, 232, and 233) developed tumors when injected intramuscularly, but when reinjected intradermally, all failed to develop tumors. One of these (11) was reinjected

twice, five months and seven and a half months after the original injection, but while it was carrying a tumor. Each reinjection was negative.

Two chickens (43 and 47) originally were injected intradermally in the left wattle. Chicken 43 later was infected and 47 developed a tumor in the wattle. When chicken 47 was reinjected three months later, intramuscularly in the breast and intradermally in the wing, the reaction was negative in these sites. The wattle tumor remained small and was present when 47 was killed five months after the original injection.

Most chickens injected with strain 13 developed tumors that grew rapidly and caused death in a relatively short time. Occasionally, a chicken was found that did not develop a tumor. Usually, chickens that did not develop a tumor at the first injection, developed one later. However, it has been noticed over several years that rarely a chicken is found that never develops a tumor even on repeated injections. Thus, it is difficult to find very many chickens that are resistant to tumors.

SUMMARY

Fowl sarcoma, strain 13, a transmissible tumor, was used to study the resistance of chickens to tumors. Most of the chickens injected in these experiments developed tumors at the site of injection that grew rapidly and caused death in a short time. Twelve chickens were obtained from 7 experiments that lived long enough for reinjection experiments. These 12 chickens were reinjected at various intervals with tumor material. Reinjections were made intramuscularly and intradermally at different sites.

Two chickens negative to the first injection developed small tumors from later injections. The other chickens that were carrying small tumors usully failed to develop tumors on reinjection. These experiments show that some chickens are resistant to tumor formation. Chickens injected with fowl sarcoma, strain 13, develop tumors whether injected intramuscularly or intradermally. Chickens carrying fowl sarcoma, strain 13, whether located intramuscularly or intradermally, are resistant to further injection with this strain.

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Observations on the Use of Sulfonamides for Calf Scours and Pneumonia

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EACH YEAR, a large number of calves are lost to the dairyman because of calf scours and pneumonia. Although a considerable amount of investigational work has been done on the etiology and pathology of these two diseases in the dairy calf, losses still persist in many localities. Before discussing the use of the sulfonamides, a brief review of past observations on these diseases will be necessary. Hagan[1] and Smith and Orcutt[2] have attributed calf scours to strains of *Bacillus coli*. Many others have confirmed these reports. Carpenter and Gilman[3] have attributed calf pneumonia to streptococci, *Corynebacterium pyogenes*, and coliform organisms. Thorp and Hallman[4] isolated streptococci of the beta type and *Escherichia communior* from cases of calf pneumonia. Recent studies by Baker[5] indicate that calf pneumonia is possibly caused by a virus. In this study, he describes the disease as pneumoenteritis.

Studies at the Florida station by Sanders[6] attribute bronchopneumonia to a strain of the Pasteurella organism and a poor environment. In a recent report, Thorp *et al.*[7] indicate that a hemophilic-like organism may be the causative agent in calf pneumonia. From this brief review, the impression is given that calf scours is probably due to a strain of the colon bacillus, but in pneumonia several different causative agents have been suggested.

Clinical Symptoms and Pathology.—The clinical manifestations of scours and pneumonia should be considered separately, but as in all diseases the symptoms and pathological changes are closely related.

The occurrence of a diarrhea and loss of appetite are observed almost simultaneously in the calf affected with scours. The diarrhea varies from a somewhat cheesy or mucoid consistency of the feces to a thin, watery type, the latter being the more severe and difficult to treat successfully. In either of these types, the feces may be black or brownish due to the diffuse hemorrhage of the small intestine. The symptoms in calf scours and the age of the calf have a great influence on the prognosis of the case. The 1- to 2-day-old calf with a severe, watery diarrhea is the most unfavorable type. Under ordinary circumstances in these cases, death usually occurs within the next one to three days. Scours which occur in calves after they are 1 week old can be dealt with more satisfactorily. In those cases which die from the severe, watery type, one finds upon autopsy a large amount of coagulated milk in the stomachs, a gastritis, and in most cases a severe, acute, hemorrhagic enteritis. The large masses of coagulated milk have been found in calves which have not taken milk for two or three days prior to death (fig. 1). When scours in its severest form occurs in a large herd, it presents a difficult problem to the veterinarian and the owner.

Calf pneumonia is most frequently observed in calves 2 to 10 weeks of age, and in some cases it may be a sequel to calf scours. The early symptoms are extreme lacrimation, a clear, watery discharge from the nose, followed by a dry cough in acute, rapidly progressing cases. These are accompanied by a rise in temperature, usually to 104.6 or 105.5 F. The development of a mucopurulent discharge from the nose is

Authorized for publication on Feb. 25, 1943, as paper No. 1166 in the journal series of the Pennsylvania Agricultural Experiment Station. Presented at the fifty-ninth annual meeting of the New Jersey Veterinary Medical Association, Trenton, N. J., Jan. 14, 1943.

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E. R. Squibb and Sons, New York, provided the sulfapyridine; Lederle Laboratories, Pearl River, N. Y., the sulfathiazole, sulfadiazine, and sulfaguanidine; and Sharp & Dohme, Inc., Glenolden, Pa., the sulfasuxidine.

accompanied by accelerated respiratory rate and marked râles, presenting a typical picture of acute pneumonia. The calf may die in five to seven days and upon autopsy, the gross lesions found are those of typical, acute bronchopneumonia which began in the anterior lobes and progressed posteriorly, involving a variable portion of the diaphragmatic lobes. Usually, the anterior lobes are in a state of grey hepatization and varying degrees of atelectasis. Some pleurisy may be present.

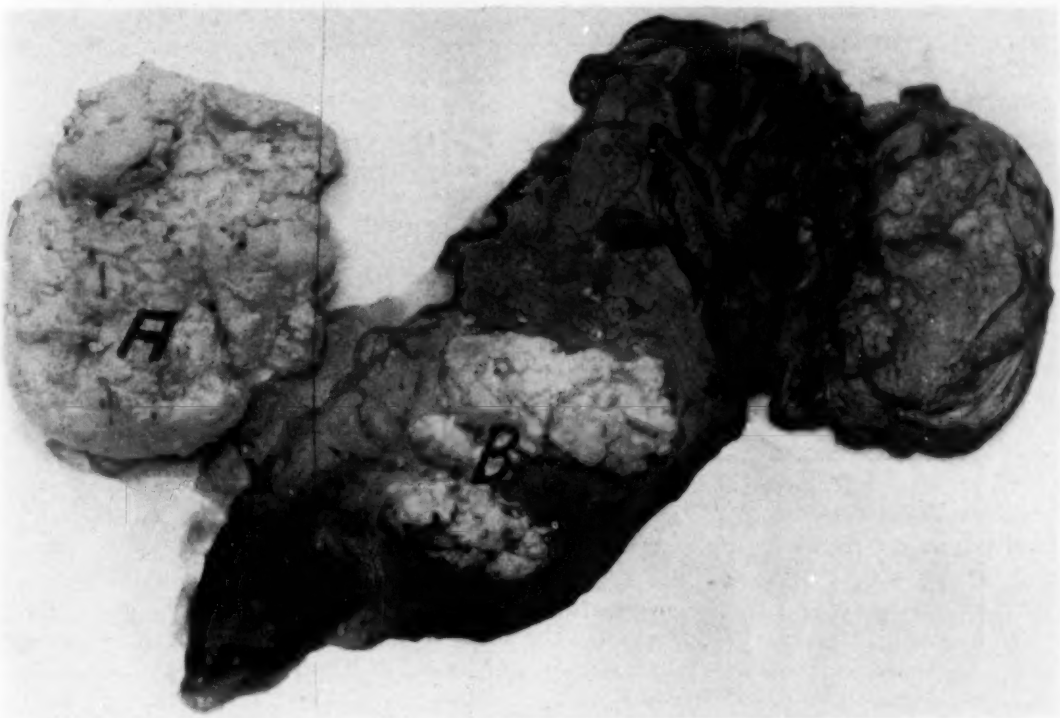

Fig. 1—Stomachs of a 3-day-old calf which died from severe scours. Note large mass of coagulated milk at A and smaller masses at B. This condition was accompanied by a severe gastritis and enteritis.

In some cases, the pneumonia becomes subacute or chronic, due to the large number of abscesses resulting from the marked necrosis and inflammatory reaction in the lung tissues.

Predisposing Factors. — The factors which contribute to the development of scours and pneumonia in a herd should be considered in relation to the etiological agents above mentioned. The apparent effect of the predisposing factors is governed to a certain extent by the virulence of the causative agents. As an example, our records show that calf scours becomes more severe the longer it remains in a given herd. Thorp *et al.*,[8] in a study of scours, showed that in herds where calf scours was a serious problem, the regulation of the intake of the dam's milk would reduce the incidence and severity of the disease. The tendency for large, coagulated masses of colostral milk to form and remain in the stomach is materially reduced by this procedure. Smith and Orcutt[2] in their work indicate that scours may develop from a local increase of *B. coli* in the digestive tract, thereby disturbing the balance between the mucous membranes and the strains of *B. coli*. Their summary is probably the best explanation of the fundamental reason for the development of calf scours. It is as follows: "The necessary conditions for such attacks are, in part, inherited defects of the digestive tract,

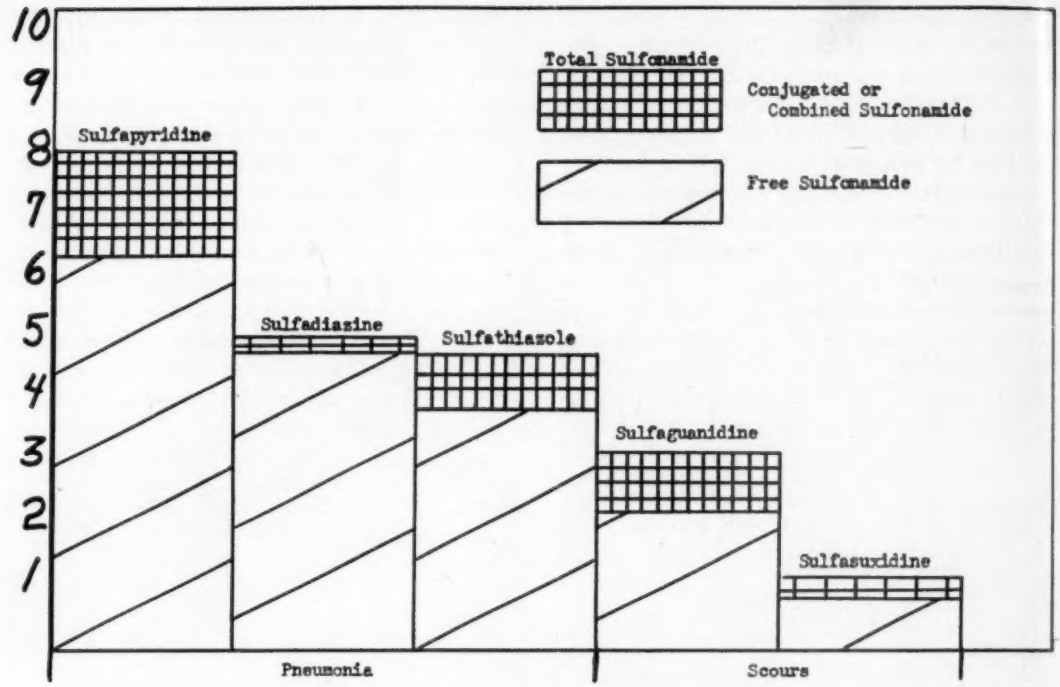

Chart 1—The average blood concentrations of the various sulfonamides obtained by giving what is considered the therapeutic dose.

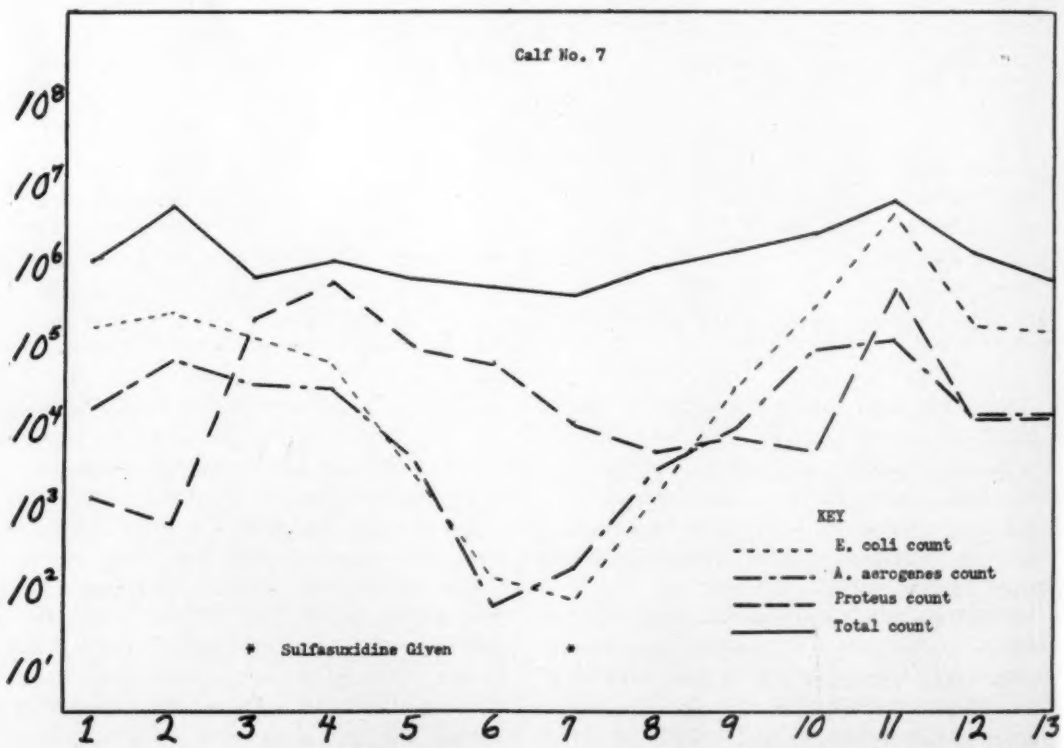

Chart 2—The effect of sulfasuxidine on the microflora of the gastrointestinal tract of the calf.

both morphological and functional, and special types of *B. coli*, resident in the herd environment."

Calf pneumonia is controlled to some extent by proper ventilation, general sanitary conditions, and the facilities for care and handling of the calves. Proper ventilation and preventing the pens from getting too damp are the most important factors in management.

As indicated by the work of Phillips and coworkers,[9] vitamins A and B are important. They claim that scours and the resultant pneumonia can be eliminated by the administration of these vitamins. The fact that vitamins A and B are essential in the calf's diet is not disputed, but we were unable to eliminate these conditions by their use. This was particularly so in the case of acute scours in the day-old calf.

TABLE I—Blood Determinations on an Experimental Calf Given Sulfasuxidine (Succinyl Sulfathiazole)*

Date	N P.N.† (Mg. %)	Urea N.† (Mg. %)	Succinyl Sulfathiazole in Blood: Total (Mg. %)	Free (Mg. %)	Combined (Mg. %)	Combined (% of total)
		Control Period				
10/22/42	26.8	5.06	...	...	...	...
10/23/42	27.6	7.18	...	...	...	...
10/24/42	30.0	12.36	...	...	...	...
		Experimental Period				
10/25/42	30.40	9.83	0.44	0.22	0.14	31.2
10/26/42	28.80	6 48	0.58	0.14	0.39	66.6
10/27/42	31.20	7.07	0.56	0.22	0.25	45.0
10/28/42	32.40	7.07	0.39	0.20	0.11	28 6
10/29/42	29.2	6.83	0.33	0.22	0.03	8.3
10/30/42	29.4	10.60	0.22	0.14	0.03	12.5
10/31/42	30.8	10.95	0.19	0.08	0.08	42.8
11/ 1/42	31.6	11.78	0	0	0	0

*Calf weighed 180 lb.; received 1 Gm. of sulfasuxidine per kg. of body weight for five days. Total daily dose divided in 3 equal doses.
†N.P.N. = Nonprotein nitrogen. Urea N. = Urea nitrogen.

Experimental Use of Sulfonamides

With the use of the various sulfonamides in human medicine, veterinarians, in practice and research, began to look for comparable applications of these compounds, particularly sulfapyridine, sulfaguanidine, sulfasuxidine, sulfathiazole, and sulfadiazine. The inclusion at this time of all, or part, of the detailed information on the sulfonamides which show promise in veterinary medicine would be superfluous, since the reports by Alfredson[10] and Millar[11] give a good review of the action and uses of the sulfonamides as they apply to our field. The exact therapeutic action of the sulfonamides in the body has not been differentiated as to whether it is a bacteriostatic or bactericidal effect. However, it is accepted by various workers that the body defenses are an important and necessary factor in the remarkable curative action of some of these drugs.

The same general experimental study was carried out on the use of these drugs in scours as in pneumonia. Briefly, this consisted of using trial dosages arrived at from the use of sulfonamides in other animal diseases, and to some extent from the human field. These trial dosages were given to normal calves of all ages. The blood, urine, and feces were examined for drug level, effect on the blood cells, and manifestations of toxicity. After the first preliminary studies were made to exclude the possibility of any toxic effects, a number of clinical cases of both scours and pneumonia were treated with the various sulfonamides. For the most part, blood studies only were made on these cases. Concurrent with this, experimental calves were obtained for the purpose of administering two, three, and four times what was considered the therapeutic dose. Blood, urine, and fecal examinations were made on these calves, as in the preliminary investigations. The determination of the drug content of the blood, urine, and feces followed the method of Bratton and Marshall,[12] modified when necessary.

Sulfapyridine was the first drug studied. As shown in an early report,[13] definitely

beneficial effects were obtained when used in calf pneumonia. It was found that there should be a reduction of the doses every two days during the course of treatment. The experimental calves showed that this drug was not so readily eliminated and that, to avoid any accumulation in tissues of the kidney, a reduction in doses should be made. The blood level of free sulfapyridine should not exceed 10 mg. per cent. In cases where toxic symptoms were produced, a rise in the leucocyte count, followed by a neutropenia and a lowering of the erythrocyte count, was observed. In both clinical and experimental work, it was found that young calves were the most subject to toxic symptoms. The dosage for sulfapyridine based on this study is given in table 4.

Sulfadiazine and sulfathiazole were studied to a limited extent. Preliminary observations on the blood, urine, and feces indicated that the calf will tolerate considerably higher doses of sulfathiazole and sulfadiazine than of sulfapyridine. Sulfathiazole is more readily eliminated from the urinary tract than sulfapyridine. An experimental calf weighing 70 lb. was given twice the therapeutic dose of sulfathiazole without the occurrence of toxic reactions. The highest blood concentration of free sulfathiazole obtained during the five-day period was 7.5 mg. per cent. Twelve cases of acute calf pneumonia in its early stage have been successfully treated with sulfathiazole. Sulfadiazine has been used for 10 cases of acute calf pneumonia with a successful recovery. In 2 additional cases of pneumonia where sulfathiazole was used and there had been a recurrence of the disease within a week, sulfadiazine was administered successfully. The blood concentrations of sulfapyridine, sulfathiazole, and sulfadiazine obtained by administering the present therapeutic dosage are shown in chart 1.

In the treating of calf scours with the sulfonamides, it should be kept in mind that investigators on the use of these drugs for intestinal diseases in both the human and veterinary field have endeavored to find an active drug which is not absorbed by the gastrointestinal tract in sufficient quantity to cause toxic manifestation, yet one which will be effective in the presence of intestinal lesions. In the course of these investigations, Woods[14] showed that para-aminobenzoic acid and like compounds had to be taken into consideration. Poth *et al.*[15] observed that those compounds which were easily hydrolyzed and resulted in readily absorbed split products gave rise to toxicity, while those not readily split by mineral acids but needing sodium hydroxide for saponification were poorly absorbed from the bowel.

Sulfanilylguanidine, or sulfaguanidine, according to early reports from human use, offered great possibilities. In an early report[16] on its use in calf scours, a large

TABLE 2—Urine Determinations on an Experimental Calf Given Sulfasuxidine (Succinyl Sulfathiazole)*

			Succinyl Sulfathiazole in Urine					
							24-hr. sample	
Date	Sp. Grav.†	Volume† (cc.)	Total (Mg. %)	Free (Mg. %)	Combined (Mg. %)	Combined (% of Total)	Total Gm.	Free Gm
			Control Period					
10/22/42	1.043	1640	.....	....	....	...	....	....
10/23/42	1.039	2500	.....	....	....	...	....	....
10/24/42	1.042	1880	.....	....	....	...	....	....
			Experimental Period					
10/25/42	1.029	3180	74.37	11.0	59.08	79.4	2.365	0.318
10/26/42	1.042	1940	125.10	18.75	99.04	79.1	2.427	0.364
10/27/42	1.040	2700	100.08	17.50	75.75	75.7	2.702	0.473
10/28/42	1.049	2020	115.37	16.0	93.13	80.7	2.331	0.323
10/29/42	1.044	2500	113.98	21.0	84.79	74.4	2.849	0.525
10/30/42	1.047	2200	61.16	13.0	43.09	70.5	1.346	0.286
10/31/42	1.029	3900	0	0	0	0	0	0
11/ 1/42	1.037	2800	0	0	0	0	0	0

*Calf weighed 180 lb.; received 1 Gm. of sulfasuxidine per kg. of body weight for five days. Total daily dose divided in 3 equal doses.

†Sp. Grav. = Specific gravity. Volume = Volume of urine in cc.

TABLE 3—Fecal Determinations on an Experimental Calf Given Sulfasuxidine (Succinyl Sulfathiazole)*

		Succinyl Sulfathiazole in Feces					
						24-hr. sample	
Date	Feces (Gm.)	Total (Mg. %)	Free (Mg. %)	Combined (Mg. %)	Combined (% of total)	Total (Gm.)	Free (Gm.)
			Control Period				
10/22/42	2722	.....	....	.....	...	.....	....
10/23/43	3625	.....	....	.....	...	.....	....
10/24/42	2722	.....	....	.....	...	.....	....
			Experimental Period				
10/25/42	2835	1278.8	27.5	1240.6	97.0	36.254	0.779
10/26/42	3625	1751.4	95.0	1619.35	92.4	63.488	3.444
10/27/42	3175	1723.6	90.0	1598.5	92.7	54.724	2.768
10/28/42	3629	1334.4	80.0	1223.2	91.7	48.425	2.903
10/29/42	4990	1390.0	110.0	1237.10	90.0	69.361	5.489
10/30/42	5670	417.0	40.0	361.40	86.7	23.644	2.268
10/31/42	4990	0	0	0	0	0	0
11/ 1/42	3402	0	0	0	0	0	0

*Calf weighed 180 lb.; received 1 Gm. of sulfasuxidine per kg. of body weight for five days. Total daily dose divided in 3 equal doses.

number of cases were successfully treated. Since that time, extensive investigations (unpublished data) have been carried out. These indicate that the dose given in the original work could be increased but very little. No evidence of conjugation of the drug was found upon examination of the feces.[17] When a therapeutic level of sulfaguanidine was administered for a period of forty days, the only changes were a reduction in nonprotein and urea-nitrogen content of the blood. Ninety-five per cent of the sulfaguanidine was recovered from the excreta when the therapeutic dosage was administered. There is more absorption from the gastrointestinal tract in the scouring calf than in the normal one of the same age. A histopathological study of the tissues removed from experimental calves receiving the same dosage showed no changes. The drug in toxic doses produced severe nephritis.

Succinylsulfathiazole, or sulfasuxidine, showed promise of being an effective drug in calf scours. Concentration of the drug in the blood, urine, and feces of 8 experimental calves has been determined. The drug levels for 1 of these calves are shown in tables 1, 2, and 3. The low blood concentration of this sulfonamide is an important factor. The therapeutic dose of 0.5 Gm. (7.7 gr.) per kg. of body weight has been increased to 1 Gm. (15 gr.) and given without toxic manifestations. The use of this drug prophylactically in the newborn calf did not decrease the incidence of scours to any great extent, but seemed to lessen slightly the severity of the disease. The effect of sulfasuxidine on the microflora of the gastrointestinal tract of the calf is shown in chart 2. There is a decided drop in the coliform organisms, but very little effect on any of the other microflora. Cultures made of the feces for the lactobacillus showed only a slight or insignificant increase. The effect of sulfasuxidine on the *Escherichia coli* and *Aerobacter aerogenes* organisms of the gastrointestinal tract is compared to that of sulfaguanidine. This and the effect of sulfasuxidine on these organisms in a case of scours is shown in chart 3.

Treatment of Scours and Pneumonia—In the early work with calf scours at this station,[7] we used various methods of treatment. Bismo-pepsol, 2 to 3 oz., and aromatic spirits of ammonia, 0.5 oz., were used in combination with anti-calf-scour serum.

TABLE 4—Present Therapeutic Dosages of the Sulfonamides for Calf Scours and Pneumonia

Drug	Size of Calf (Lb.)	Dosage (Gr. per lb.)	Desired Blood Concentration (Mg. %)
	Pneumonia		
Sulfapyridine*	Up to 70...	0.75	5 to 6
	70 to 100..	0.9	5 to 8
	Over 100 ..	1.0	5
	Scours		
Sulfaguanidine	Under 75 ..	1.0	Not to exceed 3.5
	75 and over.	2.2 to 2.5	
Sulfasuxidine	Under 100...	3.5	Blood level not readily produced
	Over 100....	3.5 to 5.0	

*Reduce dosage 0.3 gr. every two days. Sulfathiazole and sulfadiazine have the same dosage as sulfapyridine, but it is not necessary to reduce the dosage until after the third day.

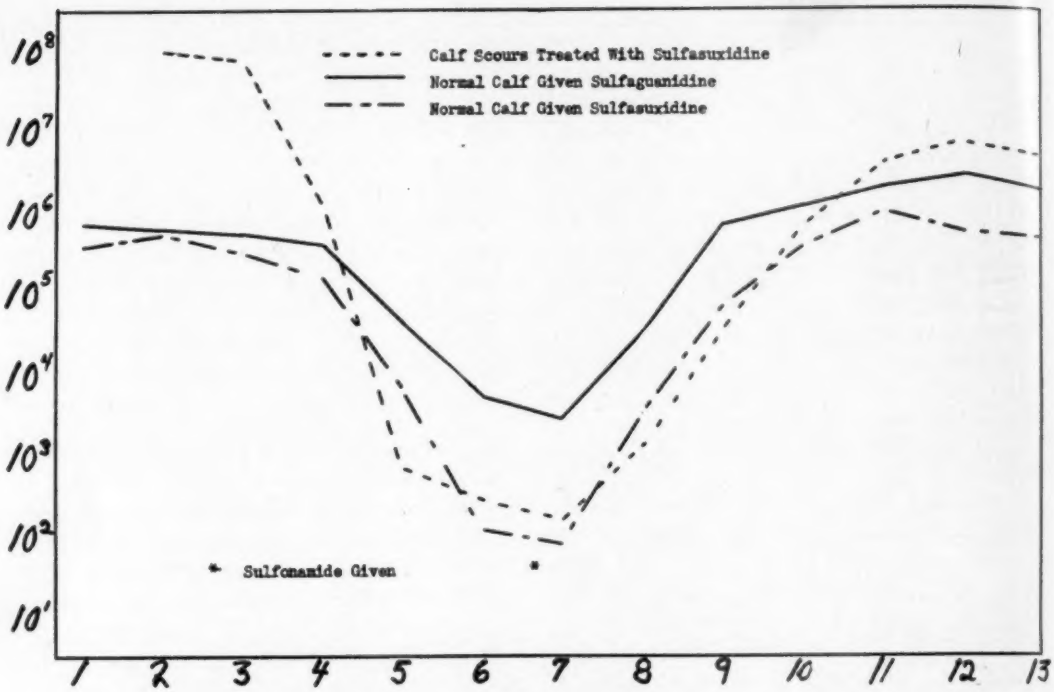

Chart 3—The effect of sulfasuxidine and sulfaguanidine on the coliform bacteria in the gastrointestinal tract of the calf.

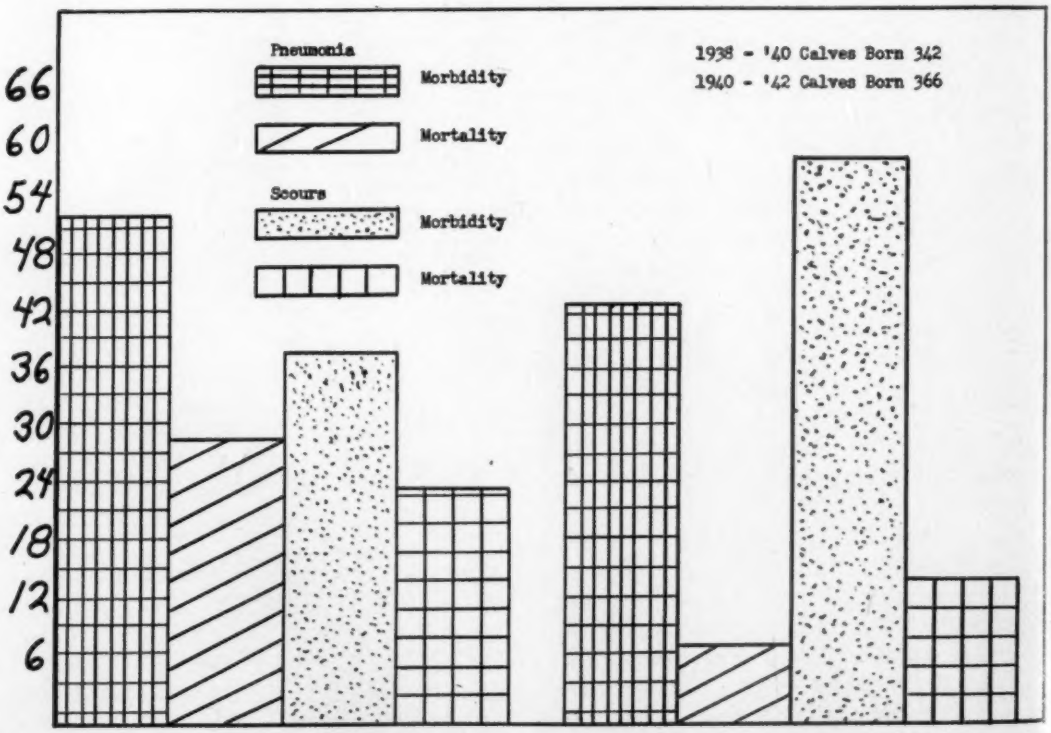

Chart 4—The incidence of pneumonia and scours from September, 1938, to September, 1940, compared to the period September, 1940, to September, 1942.

This procedure met with only fair to poor results. A specific antiserum was prepared by hyperimmunizing cows with three different strains of *B. coli* isolated *post-mortem* in calves which were about to die from scours. This specific serum gave better protection as a routine procedure. Since the number of cases of scours has been reduced, we are using another source of serum for injection at birth.

The five sulfonamides discussed in this report have shown the most promise in the treatment of scours and pneumonia of the dairy calf. In the practical application of the sulfonamides to these two diseases, one should keep in mind that if the sulfonamides are toxic, they are more so for the younger calf. Clinically, the manifestations of toxicity usually consisted of pain in the lumbar region, colic-like symptoms, and a stiffness of the limbs in severe cases. The wide use of any new sulfonamides should not be undertaken without determinations of the concentration in the blood of both clinical cases of the disease as well as in normal animals. To arrive at the proper dosage, toxicity studies should be made on a sufficient number of animals.

Sulfapyridine was used on 47 cases of acute calf pneumonia, 3 of which did not respond. Sulfathiazole was used on 12 cases and sulfadiazine on 10 cases of acute calf pneumonia. All responded to the treatment and recovered. Sulfaguanidine was used on 57 cases of calf scours, 6 of which did not respond. Sulfasuxidine was used on 27 cases of severe calf scours, 3 of which did not respond. In addition to the above clinical aspects, blood, urine, and fecal determinations were made on a comparatively large number of experimental calves. The dosages shown in table 4 are the present therapeutic amounts used, and are being used for calf scours and pneumonia. These are based on the experimental work carried out up to this time.

SUMMARY AND CONCLUSIONS

Most investigators attribute calf scours to a strain of *Bacillus coli*, and calf pneumonia to several causative agents. Recent reports indicate that calf pneumonia is probably due to a combination of a virus and bacteria.

Sulfonamides properly prescribed by a qualified veterinarian have shown definite promise in the treatment of calf scours and pneumonia.

These drugs give the best results when used early, particularly in calf pneumonia.

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The Diagnosis of Avian Neoplasia

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NEOPLASTIC DISEASES account for much loss in chickens, but the extent can not be definitely determined. There is considerable variation in the incidence of neoplastic diseases in different flocks. Among 2,304 chickens submitted to the Department of Veterinary Science at Massachusetts State College, 297 tumors were found, an incidence of 12.9 per cent.[1] It is generally recognized that tumors of lymphoid cells are more common than other types of neoplasia in chickens. Since this is true, and with lack of attempts at differentiation between lymphoid tumors and "tumors," the terms sometimes approach synonyomy. Thus, lymphoid tumors receive an onus which is not entirely deserved. A number of laboratories have been established or expanded during the past twenty-five years principally for the diagnosis of poultry diseases. The amount of material coming to some of these laboratories has so taxed the facilities that complete examinations are not always possible, and cases which appear to be neoplastic on gross examination are collectively grouped as "tumors." Practitioners of veterinary medicine frequently do not have ready access to facilities for microscopic study of neoplasia; and because of the low unit value of the individual chicken, histological examinations may never be practical. Reports of agencies conducting necropsy examinations on chickens would, however, be more valuable if an attempt were made to identify the neoplasms according to types, even though histological examination is not possible in each case. Many poultry breeders are selecting their flocks, in part, on the basis of familial incidence of tumors. Some families may be sacrificed unnecessarily if some types of neoplasia have a foundation in heredity and others do not. In doing such selective breeding it would seem desirable to maintain records on the incidence of types of tumors in families rather than only of the "tumors."

This report represents an inquiry into the problem of how accurate diagnoses of neoplasia may be when based only on macroscopic features apparent at necropsy.

In a survey on neoplastic diseases, diagnoses based on macroscopic and microscopic findings were compared in a series of cases involving 284 chickens. This study was made during a two-year period on all chickens over 6 weeks of age sent to the laboratory. Most of the birds were received from various commercial poultry farms. One group of birds was received from a flock maintained at the college for genetic study. A tentative diagnosis was made at the time of necropsy, based only on macroscopic findings. The principal factors utilized in making the tentative diagnosis were location of tumor, consistency, texture, color, general character, and distribution of lesions. Age of the bird and length of illness received minor consideration. Tissues were fixed in formaldehyde solution, sectioned, and stained with hematoxylin and eosin for histological examination and final diagnosis. Special stains were employed when required. Practical application of the results of this phase of the study is related to the accuracy of the tentative diagnosis based on macroscopic examination. The sources of error among the neoplasms encountered most frequently are discussed. To discuss each incorrect diagnosis would be time-consuming; therefore, only general statements are made. In addition to in-

Contribution 443 of the Massachusetts Agricultural Experiment Station. Presented before the Section on Poultry at the seventy-ninth annual meeting of the American Veterinary Medical Association, Chicago, Aug. 24-27, 1942.

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TABLE 1.—Correlation of Tentative and Final Diagnoses in 301 Cases of Neoplastic and Non-Neoplastic Disease Found in 284 Chickens

Final Diagnoses	Tentative Diagnoses: Lymphocytoma	Leiomyoma	Embryonal nephroma	Myelocytoma	Leucosis	Epithelioblastoma	Fibrosarcoma	Neurogenic sarcoma	Hemangioma	Fibroma	Cholangioma	Hepatoma	Histiocytic sarcoma	Myxoma	Thymoma	Chondroblastoma	Melanoma	Mesothelioma	Teratoma	Endothelioma	Granuloma	Cirrhosis	Fowl paralysis	Cyst	No diagnosis	Total	Per cent Accuracy
Lymphocytoma	*108*	1		3	1	5	11	6			1	2	3		1			1			2		6		2	153	71
Leiomyoma		*20*				1			2									1			1					25	80
Embryonal nephroma			*9*				3																	1		13	69
Myelocytoma	3			*8*			1														1				2	15	53
Leucosis	3				*11*																	1			1	16	69
Epithelioblastoma	1					*7*			2				1							1				1	2	15	47
Fibrosarcoma							*5*			1	1		1			1					1				1	11	45
Carcinosarcoma						2												1								3	0
Neurogenic sarcoma								*3*																		3	100
Hemangioma	1				1				*1*																1	4	25
Fibroma	1						2									1										4	0
Cholangioma				1								1									1					3	0
Hepatoma												*2*														2	100
Histiocytic sarcoma	2						1																			3	0
Myxoma														*2*												2	100
Thymoma															*2*											2	100
Rhabdomyoma							2																			2	0
Osteochondrosarcoma																*1*										1	100
Melanoma																	*1*									1	100
Lymphangioma						1																				1	0
Mesothelioma																		*1*								1	100
Teratoma																			*1*							1	100
Non-neoplastic diseases*	8			5	3				1		1	2														20	..
Total	127	21	9	17	16	16	25	9	6	1	3	7	5	2	3	3	1	4	1	1	6	1	6	2	9	301	
Per cent Accuracy	85	95	100	47	69	44	20	33	17	0	0	29	0	100	67	33	100	25	100	0							60.4

*Includes hepatitis, cirrhosis, granuloma, nephritis, cyst, and lymphoid hyperplasia, which were mistaken for neoplastic disease on gross examination.

formation obtained in the survey, other experiences are included and suggestions are made which would tend to eliminate many of the errors made in the series of cases reported.

There was no attempt to correlate tentative and final diagnoses while the material was being collected. It is probable, therefore, that experience gained in the first part did not materially influence the results of the latter part of the survey.

The degree of accuracy of tentative diagnoses in 301 instances of neoplastic and non-neoplastic disease was 60.4 per cent (table 1). Some of the more common types of neoplasia were recognized much more accurately than others, for example, leiomymoma (80%) and embryonal nephroma (69%) as compared with epithelioblastoma (47%) and fibrosarcoma (45%). The correlation between tentative and final diagnoses in the series of cases examined is shown in table 1.

Lymphocytoma was accurately identified on macroscopic examination in 108 of the 153 cases encountered (71%). The 45 cases which were not recognized at necropsy were confused with a wide variety of conditions. Lymphocytoma should be readily differentiated from myelocytoma. In those cases of lymphocytoma in which the infiltration is particularly diffuse, soft in texture, and pale, the presence or absence of periosteal involvement may be of assistance. Myelocytoma of an ovary which has recently been functioning has a tendency to be diffuse and to cover the retrogressing ovules, presenting a picture which, when once recognized, can usually be readily diagnosed when seen subsequently. Early cases of myelocytoma may easily be overlooked and considered as degenerative change, particularly in the liver. Imprint preparations may be used effectively as an aid in the diagnosis of myelocytoma. Despite the failure to recognize 7 of the 15 myelocytomas, this type of tumor usually has characteristics which make it readily recognizable on macroscopic examination. The majority of the cases which were incorrectly tentatively diagnosed as myelocytoma were types of liver changes including granulomas, cirrhosis, and cholangioma, many of which were considered doubtful at the time of necropsy.

Leucosis was identified with a fair degree of accuracy (69%). The 3 cases of leucosis which were thought to be lymphocytoma and the case of lymphocytoma which was called leucosis were similar in appearance. The liver, spleen, and kidneys of each were more or less swollen. The livers were quite red and showed a diffuse gray mottling. The gray-red color of the bone marrow perhaps should have offered a clue in the cases which proved to be leucosis. Similar cases may be difficult to differentiate, and examination of blood smears would be of marked assistance. An anemic appearance and watery consistency of the blood will frequently be present in cases of leucosis, and petechiae and ecchymoses often occur in the duodenum. Inflammatory and granulomatous processes in the liver may give rise to a picture easily confused with leucosis, but absence of lesions in the spleen, kidney, and bone marrow will sometimes assist in differentiation.

Although errors were made in the study, a distinction between lymphocytoma and epithelioblastoma or fibrosarcoma should be accomplished with reasonable accuracy. The difference in texture and consistency is usually marked, the latter two being denser and tougher. Age of affected individuals may also assist in differentiation because lymphocytoma occurs most frequently in birds 8 to 10 months of age, fibrosarcoma in a slightly older age group, and epithelioblastoma in older birds. Three of the 5 lymphocytomas which were tentatively diagnosed as epithelioblastomas were in the ovary and were relatively firm. Carcinoma of the ovary may be of the solid type, although more often such tumors tend to be pedunculated and more rough in appearance than lymphocytoma (fig. 1). Fibrosarcoma of the ovary is uncommon. A considerable number of fibrosarcomas were found in muscles. Failure to consider the potential widespread occurrence of lymphocytoma in individual birds was largely responsible for the incorrect designation as fibrosarcoma in several cases. Had more

consideration been given to texture and consistency in these cases and less to location, the accuracy of the tentative diagnoses should have been improved. Although lymphocytoma frequently involves several organs, isolated tumors may occasionally be found in widely separated locations such as peritoneum are involved. Carcinosarcoma and histiocytic sarcoma might also be included in this category. A firm, nodular type of neoplastic growth with extensive involvement, particularly of the ovary, peritoneum, oviduct, and pancreas, and associated with considerable ascites, proved to

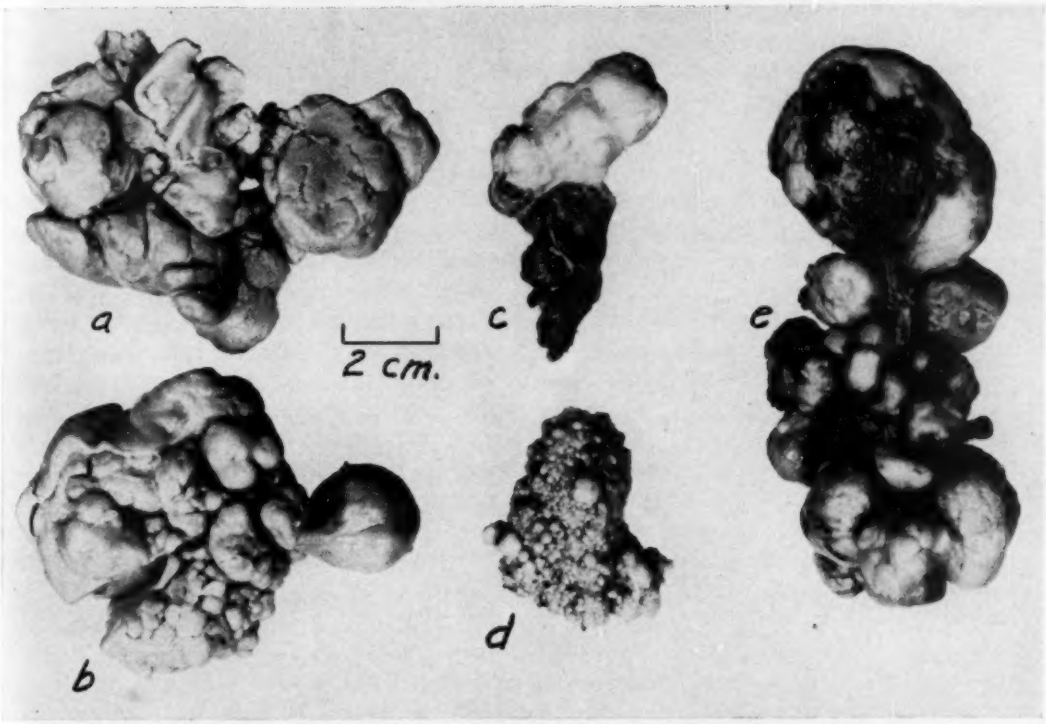

Fig. 1—Some neoplasms affecting ovaries: a. Myelocytoma; b. Lymphocytoma; c. Solid carcinoma; d. Adenocarcinoma; e. Fibrosarcoma. Consistency, texture, and color are important aids in differentiating these tumors.

the ovary, muscle, lung, bursa of Fabricius, and skin and subcutis. The color of lymphocytoma may vary considerably, particularly from gray-white to red-gray and the color of fibrosarcoma, from gray-white to gray-yellow; the color of epithelioblastoma is quite variable. The neoplasms which were incorrectly tentatively identified as fibrosarcoma, but were neither fibrosarcoma or lymphocytoma, were mostly of connective tissue origin. Although no difficulties are apparent in the results being reported, there might be considerable difficulty in differentiating between fibrosarcoma and epithelioblastoma, particularly when abdominal organs such as the pancreas, ovary, and be either epithelioblastoma, carcinosarcoma, or fibrosarcoma.

Leiomyoma was the type of neoplasm second in frequency. Only one incorrect diagnosis of leiomyoma was made. This was a tumor mass located at Meckel's diverticulum, which proved to be lymphocytoma. The leiomyomas occurring in the ventral ligament of the oviduct were readily recognized on gross examination. Those which were not recognized at necropsy occurred as concomitant tumors and were not given sufficient study or were considered a part of the principal tumor, either epithelioblastoma or carcinosarcoma.

No tentative diagnosis of embryonal

nephroma proved to be incorrect. Three of the 4 which were not recognized were confused with fibrosarcoma, and the fourth was thought to represent a cystic kidney. Embryonal nephromas are extremely variable in their gross characteristics. Size, color, character, and texture are variable. It would, however, seem relatively safe to consider any encapsulated tumor arising from the kidney as embryonal nephroma, even though the attachment to the kidney is relatively scant, when other organs are free from neoplasia.

Six cases which were tentatively identified as neurogenic sarcoma proved to be lymphocytoma. Two of these were located medial to the eye, 2 were in the region of the lumbo-sacral plexus, 1 was adjacent to the brachial plexus and dorsal root ganglia, and 1 was in the tibial region extending from the lumbo-sacral plexus. Neurogenic sarcomas are usually glistening white and have a firm texture. Some are encapsulated and the relation to nerves assists in identification. Those which are not encapsulated and are invading the surrounding tissues are more difficult to identify accurately. These tumors were found principally in birds less than 6 months of age.

Differentiation between neoplastic and non-neoplastic processes is usually considered to be relatively easy. Some difficulties, however, may arise. Examinations for fungi, bacteria, and parasites may be of assistance, although both granulomas and neoplasia may exist in the same bird. Secondary infection in a tumor may make recognition difficult. Numerous cases which were considered to be non-neoplastic at necropsy were included for histological examination and found to be granulomatous or inflammatory. However, of non-neoplastic diseases, 20 cases were thought to be neoplastic. Inflammatory and neoplastic processes in the proventriculus, liver, and kidney may be particularly difficult to differentiate. The condition of other organs may be of assistance in differentiating between lymphocytoma and proventriculitis. No case of lymphocytoma was observed in which only the proventriculus was involved. Cholangioma, cholangitis, and cirrhosis of the liver may have similar features. The kidneys are generally more firm in chronic nephritis than in lymphocytoma of the kidney.

DISCUSSION

Characteristics of tumors may be quite variable. In some cases there may be as much variation within a type as between types. Identification on macroscopic examination may be particularly difficult when the characteristics are at variance with those typical for a particular tumor. Many of the tentative diagnoses were considered questionable when made and were later found to be erroneous. Several of the incorrect diagnoses were the result of failure to recognize some cases of concomitant neoplasia on initial examination. Some of the errors were due to the finding of neoplasia on microscopic examination which were not suspected at necropsy. The number of neoplasms which were overlooked cannot be determined. All material was carefully examined before being rejected as free from neoplasia. Postmortem changes may alter tissues to the extent that the process is not readily recognized, but in such a case the same difficulty would affect histological examination.

It should not be inferred that the diagnosis of avian neoplasia from macroscopic examination alone is recommended. It must be recognized, however, that practicing veterinarians and some laboratories do not always have time or facilities for microscopic examinations of all cases. It is believed that reports from these agencies would be more useful if an attempt was made to identify the tumor type, even on macroscopic examination alone. A reasonable degree of accuracy in recognizing the more common neoplasms should be attained if careful attention is given to each case. The accuracy could be checked and perhaps improved by conducting a series of check examinations from time to time. Preparation of sections on a freezing microtome might be of particular value in such check examinations. All doubtful cases should be examined histologically.

SUMMARY

Macroscopic and microscopic diagnoses were compared during the collection and study of spontaneous neoplasms in chickens. The general order of correctness of macroscopic diagnoses was 60.4 per cent in 301 cases. The sources of error in the incorrect designations of the more common neoplasms are discussed.

Inherited Resistance to Brucellosis in Inbred Berkshire Swine

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IN 1922, the California Agricultural Experiment Station began experimental inbreeding of Berkshire swine. The results to 1933 have been reported by Hughes.[1] In 1929, there was a serious outbreak of swine abortion; nearly half the university herd reacted to the blood test for brucellosis. Since the effect of inbreeding on birth weights and litter size was an objective in the experiment, the presence of brucellosis constituted a serious variable. In 1931, therefore, a nucleus for continuing the experiment was formed by saving one boar and two gilts, the offspring of inbred sows, and negative to the blood test for brucellosis in spite of natural exposure. In this way, it was hoped to eliminate one variable which had interfered with birth weights and litter size besides causing breeding difficulties. Since that time, the program has continued uninterrupted, and the herd has remained free from brucellosis.

During investigations on brucellosis of swine the authors[2, 3, 4] found resistance in grade sows from purebred stock originating in the university herd. To judge from the evidence, the resistance was genetic, being transmitted to the progeny. Since the ultimate object was to establish resistance in commercial herds, the condition was sought in purebred stock. This paper reports the results of the attempt and gives data strongly suggesting that the inbreeding of this particular group of Berkshires has established a strain resistant to brucellosis.

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EXPERIMENTAL

The methods of determining resistance or susceptibility were described in previous publications.[2, 4] Pigs about 12 weeks old were given suspension of virulent *Brucella suis*, orally. During the next three months, they were blood-tested periodically. The tube technique in dilutions of 1 : 25, 1 : 50, 1 : 100, and 1 : 200 was used. Specific agglutinins in the blood stream indicated susceptibility; their absence, resistance. The first attempt was made on the Poland China breed. In an unselected group of 33 pigs used as controls in the original work,[2] a purebred Poland China gilt appeared to be resistant. Seven young Poland China boars from the university herd were tested, with the results shown in table 1. Boar 2 was apparently resistant, a supposition confirmed by subsequent tests and attempts to infect. The gilt was mated to the boar. Of the 20

TABLE 1—Results of Attempts to Infect a Group of Poland China Boar Pigs

PIG	INOCULUM	BLOOD TITERS* 23 DAYS	25 DAYS	45 DAYS
2	5 cc. *Br. suis* per os	+ I — —	+ — — —	— — — —
3		+ + I —	+ + + +	
16		+ + I —	+ + + +	
20		+ + + I	+ + + +	
21		+ + — —	+ + — —	
22		+ + — —	+ + — —	
29		+ + + —	+ + + +	

*Dilutions—1 : 25, 1 : 50, 1 : 100, 1 : 200.

progeny tested thus far, 18 proved resistant, whereas 2 were undetermined. Though the latter reacted after exposure, the titer was transitory, not persisting as in a true susceptible.

Because inbreeding has been practiced with the university Berkshires, and because

TABLE 2—Results of Attempts to Infect a Group of Inbred Berkshire Pigs

Pig	Inoculum	Blood titers* 22 days	49 days	79 days
1	5 cc. of a suspension of *Br. suis per os*	— — — —	— — — —	— — — —
7		— — — —	— — — —	— — — —
9		— — — —	— — — —	— — — —
10		— — — —	— — — —	— — — —
50		— — — —	— — — —	— — — —
51		— — — —	— — — —	— — — —
53		— — — —	— — — —	— — — —
66		— — — —	— — — —	— — — —
69		— — — —	— — — —	— — — —
70		— — — —	— — — —	— — — —
72		— — — —	— — — —	— — — —
79		— — — —	— — — —	— — — —
81		— — — —	— — — —	— — — —
83		— — — —	— — — —	— — — —
90		— — — —	— — — —	— — — —
93		+ I — —	— — — —	— — — —
99		— — — —	— — — —	— — — —

*Dilutions—1 : 25, 1 : 50, 1 : 100, 1 : 200.

it began with stock perhaps naturally resistant, we suspected that the entire Berkshire herd may be uniform in its reaction to the agglutination test.

An initial test on 4 pigs from the spring 1942 crop showed all to be resistant. To obtain more significant data, 14 pigs were selected from a total of 28, from the fall 1942 crop. Removed from the university herd about two weeks after weaning, they were placed with the herd of the division of veterinary science and were immediately given a suspension of *Br. suis*, orally. The results (table 2) indicate complete resistance in the group.

Controls consisted of 34 grade pigs, the progeny of crossbred sows from the university herd. They were infected in the same manner as the purebreds. Their reactions (table 3) show a marked variation: 13 remained negative in all dilutions, 10 were positive at 1 : 100 or over, and about 50 per cent of the remaining intermediate reactions reverted to negative.

Discussion

The foregoing results indicate the possibility of developing strains of swine resistant to brucellosis. Undoubtedly, the resistance was natural and not acquired: The pigs were raised in an environment free from the infection for at least ten years, and the attempt to infect came immediately after removal to quarters where natural infection was possible. The Poland China herd in which susceptibility existed was to some extent inbred. The status of the original stock, however, was not established. On the other hand, in the Berkshires the original 2 gilts and 1 boar were apparently resistant since they had been under severe natural exposure and showed no evidence of having acquired the infection. The results in the Poland China pigs, therefore, serve as an additional control group.

The resistance now existing in this strain of Berkshires has been, no doubt,

TABLE 3—Results of Attempts to Infect a Group of Grade Pigs (Controls)

Pig	Inoculum	Blood titers* 19 days	31 days	48 days
283	5 cc. of a suspension of *Br. suis per os*	— — — —	— — — —	— — — —
284		— — — —	— — — —	— — — —
289		— — — —	— — — —	— — — —
291		+ + — —	+ — — —	+ + — —
295		— — — —	— — — —	— — — —
296		— — — —	— — — —	— — — —
297		— — — —	+ I — —	+ + + +
302		I — — —	— — — —	+ + + +
310		— — — —	— — — —	I — — —
316		I I — —	+ + I —	+ + + I
317		— — — —	+ + — —	+ + + +
321		+ + — —	+ + I —	+ + — —
322		— — — —	— — — —	— — — —
329		+ — — —	— — — —	— — — —
333		— — — —	— — — —	— — — —
335		+ — — —	I — — —	+ + — —
336		+ + — —	+ — — —	— — — —
337		I — — —	— — — —	+ + — —
338		— — — —	— — — —	+ + — —
339		— — — —	— — — —	— — — —
341		+ I — —	+ I — —	+ — — —
342		I — — —	— — — —	— — — —
343		+ — — —	+ I — —	+ — — —
345		+ — — —	I — — —	+ + + +
346		I — — —	I — — —	+ + + +
348		+ — — —	I — — —	+ + + —
351		+ — — —	+ + — —	+ — — —
352		— — — —	— — — —	— — — —
353		— — — —	+ — — —	+ + I —
356		+ — — —	— — — —	+ + + +
359		— — — —	+ — — —	+ I — —
362		— — — —	— — — —	— — — —
363		— — — —	+ + I —	+ + — —
372		+ + — —	+ I — —	+ + + +

*Dilutions—1 : 25, 1 : 50, 1 : 100, 1 : 200.

inherited from the original resistant stock. This is further substantiated to a limited extent by the creation of resistant Poland China stock through resistant X resistant matings. The development of resistant lines would be of immediate value to the swine breeding industry, where the disease

causes heavy losses, and also of interest from the standpoint of public health. These and other results cited from this laboratory lead us to believe that brucellosis in swine can be controlled under average field conditions by the use of lines that are genetically resistant to *Br. suis.*

From the standpoint of controlled experiments on brucellosis where the pig is the experimental host, it is becoming more and more apparent that a homozygous susceptible strain of swine should be used. The marked variation in reactions to the agglutination test of the animals shown in table 3 can be attributed solely to genetic heterogeneity of the swine population tested. If the genotype for resistance is not taken into account, considerable confusion may result. Most investigators assume that, as a species, hogs are susceptible to *Br. suis,* and the validity of the agglutination test may be questioned if an animal fails to show agglutinins after repeated experimental exposure. The difficulty, however, was not in the agglutination test, but due to differences in genotype for resistance and susceptibility in the swine population.

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Mycobacterial Rapid Agglutination Antigens and Their Diagnostic Value in Tuberculosis of Fowl

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A PROCEDURE for the detection of mycobacterial agglutinins that is uniformly reproducible is not available. This situation is a result largely of certain features of the behavior of suspensions of mycobacteria. The methods that are employed commonly for the preparation of cellular suspensions or tube agglutination test antigens from other microörganisms and from the majority of the species or the strains of mycobacteria yield unstable suspensions of the latter bacteria. The instability is manifested by the occurrence, singly or in combination, of varying degrees of spontaneous agglutination and of sedimentation of the suspended cells.

That part of this report concerning the preparation and usefulness of rapid or concentrated test antigens resulted from efforts to obtain a stable antigen for agglutination tests. This was desired in the course of studies on the antigenic character of certain mycobacteria. In addition, an antigen was desired for use in determining the presence of seroagglutinins as a possible diagnostic aid for tuberculosis in pigeons. The availability of rapid test antigens prepared from *Mycobacterium avium* organisms prompted a test of the value of the antigens in the detection of specific seroagglutinins, and, hence, in the diagnosis of tuberculosis in chickens.*

The method of preparation and the characteristics of concentrated antigens prepared from various strains of mycobacteria, and a consideration of the usefulness of *M. avium* antigens as diagnostic agents for tuberculosis in pigeons and chickens are presented in this report.

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*For a consideration of the value of other types of test antigens in the diagnosis of tuberculosis in chickens by the agglutination test, see the monograph by W. H. Feldman: Avian Tuberculosis Infections. Williams and Wilkins Co., Baltimore, (1938): 135.

MATERIALS AND METHODS

Preparation of Concentrated Antigens.—The strains of mycobacteria used in the preparation of concentrated antigens included 3 strains of *M. avium*, 2 strains each of *Mycobacterium tuberculosis hominis* and of *Mycobacterium tuberculosis bovis* and 1 strain of the vole bacillus.*

The respective strains of the various mycobacteria were cultured on an egg yolk-agar medium. Blake-type bottles were used as culture containers. Cultures were incubated at 37.5 C. for approximately three weeks, or until maximal growth was obtained. The bacilli were dislodged from the agar surface and suspended in 20 to 30 cc. of 0.85 per cent solution of sodium chloride. This was facilitated with the aid of gentle rubbing of the bacterial growth by a piece of flexible rubber tubing attached to the end of a glass rod. The bacteria were killed by exposure to a temperature of 65 to 75 C. for half an hour. The suspension of bacilli was homogenized by agitation in the presence of glass beads for half an hour in a power shaking machine. The bacilli were separated from the bulk of the suspending fluid by centrifugation, and the supernatant fluid was discarded. A suspension of the bacilli was then prepared with a sterile 0.85 per cent solution of sodium chloride to which were added 0.5 per cent phenol and 0.4 per cent gelatin, as recommended by Donham and Fitch[1] for the preparation of Brucella antigens. The suspension was homogenized by agitation, as before, and any remaining clumps of bacilli were removed by filtration through glass wool. The concentration of bacteria in the suspension was adjusted to 10 per cent by volume by the use

*A subculture of the vole bacillus was supplied by Dr. W. C. Brooks, Johns Hopkins Medical School, Baltimore, Md.

of a volumetric centrifuge tube described by Donham and Fitch.[1] The hydrogen-ion concentration was made to conform to the pH 7.4 to 7.6 by the addition of the necessary amount of normal solution of sodium hydroxide.

Method of Use of Antigens.—The antigens were used in a manner essentially similar to the procedures that are employed with other plate or rapid test antigens. The tests were conducted at room temperature of from 25 to 30 C., and the temperature of the materials used in conducting the tests corresponded to that of the room. Pipettes of 0.2 cc. capacity, graduated in 0.01 cc., were used for measuring the quantities of serum to be tested. The quantities of whole serum that were tested were 0.2, 0.1, and 0.05 cc. To test smaller amounts of the serum, it was appropriately diluted and 0.1 and 0.05 cc. of each dilution were tested.

The measured amounts of whole or diluted serum were placed on a conveniently illuminated glass plate, and to each was added 0.03 cc. of the antigen. The individual sets of test materials were mixed by stirring with a toothpick, beginning with the smallest test quantity of serum, and by rotation of the plate. Significant reactions occurred quickly, or within thirty seconds.

Aging of the antigens for periods of four months was not observed to affect adversely the usefulness of the antigens.

Agglutination Tests with Serums of Tuberculous Pigeons.—The serums of 14 tuberculous pigeons were examined for mycobacterial seroagglutinins. The demonstration of these antibodies was attempted with 2 concentrated antigens prepared from individual strains of *M. avium.** Each of these pigeons was tested for hypersensitivity to tuberculin by the intracutaneous injection of 0.03 to 0.05 cc. of avian tuberculin at the dorsal margin of the vent. The reactions were read after forty-eight hours. The birds were killed at this time for necropsy. The regions of tissue into which the tuberculin had been injected were fixed in 10 per cent solution of formalin and sections stained with hematoxylin and eosin were prepared for histologic examination.

Agglutination Tests with Chicken Serums.—Specimens of serums were obtained from 50

TABLE I—Significance of Mycobacterial Seroagglutinins in Comparison with Other Methods for the Diagnosis of Tuberculosis in Chickens

Chicken No.	Titer* of Seroagglutinins	Degree of Reaction to Tuberculin	Days until death†	Postmortem Evidence of Tuberculosis	
				Pathologic	Bacteriologic‡
1	0.001	+1	12 died	Extensive lesions	Positive
2	0.005	+2	71 died	Extensive lesions	Not done
3	0.005	Negative	4 died	Extensive lesions	Not done
4	0.005	Negative	54 died	Extensive lesions	Not done
5	0.005	Negative	63 died	Extensive lesions	Not done
6	0.01	Negative	29 died	Extensive lesions	Not done
7	0.01	+3	8 died	Extensive lesions	Not done
8	0.01	+3	63 died	Few tubercles in spleen, liver, and one lung	Not done
9	0.05	+2	30 killed	Miliary tubercles in liver; few tubercles in spleen	Not done
10	0.05	+2	30 killed	2 large caseous foci in spleen; 1 small tubercle in one lung	Not done
11	0.05§	Negative	23 killed	No demonstrable lesions	Negative
12	0.05§	Negative	23 killed	No demonstrable lesions	Negative
13	0.1	+3	30 killed	1 tubercle of microscopic dimensions in liver	Positive
14	0.2	+3	29 died	No demonstrable lesions	Negative

*Fraction of a cc. (up to 0.2 cc.) of serum capable of causing complete agglutination of 0.03 cc. of antigen.
†From time of conducting initial clinical tests.
‡Culture of emulsion of liver and spleen.
§No agglutinins were demonstrable in serum obtained three weeks later.

*One of these strains of *M. avium* was isolated from a tuberculous submaxillary lymph node of a swine, and the other from a tuberculous chicken.

chickens of a farm flock in which tuberculous chickens were known to have been present. Agglutination tests were conducted with the serum. The chickens were also tested with tuberculin by the intracutaneous wattle test of Van Es and Schalk.[3] Chickens whose serum gave a positive agglutination reaction, or which were hypersensitive to tuberculin, were secured for further study of the serum agglutinins and to determine the character of the tuberculous infection.

Laparotomy was performed on 21 of the chickens, in order to observe the condition of a portion of the liver.* Those chickens in which no lesions were visible by this procedure were killed within four weeks from the time of the initial tests to determine by further examination the extent of the disease. Those in which lesions were evident in the liver at laparotomy were examined further at the time of death from natural causes. In all cases in which the gross evidence of tuberculous disease was minimal or absent at the time of necropsy, portions of the spleen, liver, and lungs were removed for histologic examination, and a composite emulsion of portions of the liver and spleen was prepared for cultures for avian tubercle bacilli. The chickens represented in the group studied were older than 1½ years. The majority were of the Rhode Island Red breed; a few were White Wyandottes.

Serum was obtained for agglutination tests from 20 aged chickens of a flock in which tuberculosis has never been demonstrated. The standard tuberculin test was also conducted.

Agglutinin Absorption Studies.—The agglutinin concentration of the available serums of 3 of the naturally infected chickens was sufficient for agglutinin absorption tests. The mycobacteria used in attempts to remove the agglutinins were 2 strains each of human, bovine, and avian tubercle bacilli and 1 strain of the vole bacillus. The procedures recommended by Krumwiede, Cooper, and Provost[3] were followed in the performance of these tests, with the exception that concentrated antigens were used in the final tests.

RESULTS

Antigen Characteristics. — Concentrated antigens prepared from each of 2 strains of *M. avium* have proved useful for conducting rapid agglutination tests. The antigen prepared from 1 strain of *M. avium*, isolated from a case of spontaneous tuberculosis in a pigeon, was useless for test purposes because of spontaneous agglutinability of the cells. Similarly, this property was shown by the antigens prepared from the strain of the vole bacillus and the strains of human and bovine types of tubercle bacilli. No differences have been detected in the test sensitivity of the 2 antigens, prepared from the 2 strains of *M. avium*, with which the tests reported here were conducted.

Tests of Serums of Tuberculous Pigeons.—Complete agglutination of 0.03 cc. of the antigens occurred with the serums of 4 of 14 tuberculous pigeons. Reactions were obtained with 0.2 and 0.1 cc. of the serum. No differences of recognizable significance regarding the type* of pigeons or tuberculous disease were present between the 4 pigeons that had detectable seroagglutinins and the 10 without detectable seroagglutinins. Studies of pathogenicity and reactivity to homologous tuberculin test with chickens, rabbits, and guinea pigs show that the acid-fast bacteria isolated from 1 pigeon in the agglutination reactor group and 1 pigeon in the nonreacter group were *M. avium*.†

Occurrence and Significance of Mycobacterial Agglutinins in Chickens.—Twenty-two of the 50 chickens of the tuberculous farm flock reacted to the agglutination or to the tuberculin test. The serums of 14 of the 22 chickens gave an agglutination reaction. Sixteen of the chickens reacted to the tuberculin test. Studies conducted *post mortem* revealed evidence of tuberculous infection in 16 chickens. Tables 1 and 2 give the pertinent details of the results of these observations.

The agglutinin titers as recorded in table 1 were confirmed by one or more subse-

*This procedure was employed for the purpose of procuring for another experiment a group of chickens in which information could be available concerning the presence in the living bird of tuberculous lesions.

*Three of the reactor pigeons were common pigeons, and 1 was a member of the genus *Columba guinea*. The nonreactors were of the following types: common pigeons, 3; *C. guinea*, 5; almond pigeon and silver bar pigeon, 1 each.

†Avian tuberculin, administered intracutaneously, failed to incite a local reaction in any of the pigeons.

quent tests in all but 2 instances. Serum obtained from chickens 11 and 12, three weeks after the initial observation, was without demonstrable agglutinins. Neither of these birds was hypersensitive to tuberculin and no evidence of tuberculous infection was demonstrated by studies conducted *post mortem*. The significance of the initial agglutination reaction is obscure. Further, as shown in table 1, marked agglutination responses were obtained with serums from chickens 3, 4, 5, and 6, in which tuberculin failed to elicit a positive local response. The physical condition of these chickens at the time of the first examination was from poor to fair in 3 instances and good in 1 instance. Their deaths occurred from natural causes four, twenty-nine, fifty-four, and sixty-three days following the initial tests. All showed generalized lesions of tuberculosis. Chicken 14 gave the lowest response to the agglutination test and a marked reaction to tuberculin; yet no evidence of tuberculous infection was demonstrated by the postmortem studies.

TABLE 2—Status of Tuberculous Infection in Chickens Hypersensitive to Tuberculin but Without Demonstrable Seroagglutinins

Chicken No.	Tuberculin Test Results	Postmortem Evidence of Tuberculosis	
		Pathologic	Bacteriologic*
15	+3	1 large tubercle in spleen	Not done
16	+2	1 small tubercle in liver	Positive
17	+2	2 small tubercles in liver	Negative
18	+2	1 small tubercle in one lung	Negative
19	+3	No demonstrable lesions	Negative
20	+2	No demonstrable lesions	Positive
21	+2	No demonstrable lesions	Negative
22	+1	No demonstrable lesions	Negative

*Culture of composite emulsion of liver and spleen.

The 8 chickens whose serums in amounts as large as 0.2 cc. did not cause any agglutination of 0.03 cc. of the concentrated antigens gave variable degrees of reaction to the tuberculin test, as shown in table 2. Three agglutination tests conducted subsequent to the initial one likewise failed to show any reactors. Gross and microscopic changes of tissue indicative of tuberculosis were demonstrated in 4 of the 8 chickens. In each instance, the disease was limited to one organ, and in 2 of these instances, tubercle bacilli were not cultivable from an emulsion of portions of the liver and spleen. In chicken 20, no lesions of tuberculosis were demonstrated; yet a culture of *M. avuim* was obtained from the emulsion of the liver and spleen. No evidence of tuberculous infection was demonstrated *post mortem* in 3 cases.

Of the 16 chickens in which concrete evidence of tuberculous infection was demonstrated, the agglutination test failed to detect 5 and the tuberculin test failed to detect 4.

All of the 20 serums tested, from as many chickens from the nontuberculous flock, were without agglutinating power for the *M. avium* antigens. None of these chickens reacted to the tuberculin test.

Agglutinin Absorption Study.—The agglutinating properties of the serums of 3 chickens (2, 4, and 5) were removable by exposure to cells of *M. avium*, but not by strains of human, bovine, and vole tubercle bacilli. This finding is in accord with the observations of Wilson,[5] with the exception of the finding regarding the vole bacillus, which he did not study.

COMMENT

The method described for the preparation of concentrated cellular antigens of mycobacteria appears to be applicable to certain strains of *M. avium*. Limited observations indicate that the *M. avium* antigens are of value in detecting the agglutinins produced in chickens in response to heterologous strains of the same species. The use of the antigen described nullifies certain factors of instability, especially cellular sedimentation, possessed by the microörganism in less concentrated suspensions.

Failure of the cells of the antigens to be agglutinated by the serums of 10 of 14 pigeons infected naturally by *M. avium* is

difficult of interpretation. A comparison of breed, age, sex, and physical condition of the pigeons, together with the extent and character of the disease, failed to reveal any significant differences among the pigeons.

In flocks of chickens comparable to those studied, it would appear that the agglutination test would be of nearly equal reliability to the tuberculin test in detecting tuberculous flocks of chickens and even tuberculous members within a flock. The concentration of the agglutinins present may bear a direct relation to the extent of the disease. Extensively tuberculous chickens, which are a tremendous hazard to the contamination of premises with tubercle bacilli and which are often anergic to tuberculin, were detectable in this study by the agglutination test.

The antigen would appear to be of use in testing samples of serum sent to central laboratories for agglutination tests for pullorum disease, as an additional aid to the detection and eradication of avian tuberculosis infections.

The results of the agglutinin absorption tests show that the agglutinating properties of the serums tested were due to specific *M. avium* agglutinins.

SUMMARY

Concentrated suspensions of each of 8 strains of mycobacteria were prepared for use in rapid tests for mycobacterial agglutinins. Suspensions that were sufficiently stable for agglutination test antigens were obtained from each of 2 strains of avian tubercle bacilli. A limited number of tests were made to determine the usefulness of the antigens in detecting seroagglutinins and their value as a diagnostic aid for tuberculosis in pigeons and chickens. The serums of 4 of 14 pigeons gave a positive reaction by the agglutination test. Fifty chickens of a farm flock were tested by the agglutination and by the tuberculin test; 22 reactors were obtained. Evidence of tuberculosis was demonstrated in 16 chickens. The agglutination test detected 11 of the infected chickens, and the tuberculin test detected 12. Approximately an equal number, also, that reacted to either test were without demonstrable lesions or other evidence of tuberculous infection. These data support the following conclusions:

1) Rapid or concentrated antigens that are suitable for agglutination tests can be prepared, by the procedure described, from certain strains of *Mycobacterium avium*, but not from human, bovine, or vole types of tubercle bacilli.

2) Concentrated cellular antigens of *M. avium* are useful for the detection of mycobacterial seroagglutinins produced in chickens in response to heterologous strains of the bacterium.

3) Evidence is presented to show that detectable mycobacterial agglutinins may be absent in chickens that have minimal tuberculous disease and in tuberculous pigeons, yet the agglutination test may have a reliability comparable to the tuberculin test in the diagnosis of tuberculosis in these species.

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